The Journal of Laboratory and Clinical Medicine

VOL. X ST. LOUIS, MO., OCTOBER, 1924 NO. 1

CLINICAL AND EXPERIMENTAL

A STUDY OF THE SPECIFICITY OF THE KOLMER COMPLEMENT-FIXATION TEST FOR SYPHILIS*

BY JOHN A. KOLMER, M.D., D.SC., AND EDWARD STEINFIELD, M.D.

IN all probability the complement-fixation test for syphilis serves its most useful purpose in the diagnosis of the disease in its clinically latent and tertiary stages, as a guide in treatment and as one criterion of cure. In the primary stage it may, of course, aid in the diagnosis of clinically atypical infections when employed along with dark-field examinations for *Spirochete pallida* and likewise confirm the clinical diagnosis in the secondary stage and greatly aid in the diagnosis of atypical cases at this stage of the disease, but we believe that most physicians will agree with us that the complement-fixation test is, or, at least should be, of most service as an aid in the diagnosis of the clinically obscure, doubtful and unsuspected cases of acquired and congenital syphilis in which even a most careful and exhaustive history and physical examination may yield inconclusive and doubtful results.

The very nature of the pathologic and immunologic changes in cases of chronic and latent cases of syphilis demands that the complement-fixation test shall be as sensitive as consistent with specificity to render its best service as an aid in diagnosis, as a guide in treatment and as a criterion of cure. In the first place there are reasons for believing that the antibody-like substance occurring in the blood and spinal fluid responsible for complement fixation in syphilis is a product of the lymphocytes and other cells in more or less direct contact with *Spirochete pallida* in the tissues rather than a product of stimulation of the bone marrow and other distant antibody producing

*From the Department of Pathology and Bacteriology of the Graduate School of Medicine of the University of Pennsylvania and the Research Institute of Cutaneous Medicine, Philadelphia.

Read before the Third Annual Meeting of the American Society of Clinical Pathologists, June 5-7, 1924.

organs by a diffusible antigen as occurs in most other infections. There are no reasons at all for regarding this antibody-like substance as a true antibody in the sense of being destructive for *Spirochete pallida*; rather it is a by-product of cellular stimulation or degeneration rendering the complement-fixation reaction in syphilis a measure of the degree of infection rather than of the degree of immunity. If, however, the strength or degree of complement fixation in syphilis with lipoidal extracts is an index of the degree of spirochetic activity, there are numerous instances in which the reaction is quite marked in cases without coincident clinical evidences of activity, but it is to be kept in mind that the signs and symptoms of chronic syphilis are due largely to the location of the spirochetes and the degree of involvement of physiologically important tissues. For example, a relatively few spirochetes in the posterior columns of the spinal cord may be sufficient for the production of marked signs and symptoms of locomotor ataxia; the numbers of parasites may be indeed too few to produce sufficient of the antibody-like reagin to yield positive complement-fixation reactions and an equal number of spirochetes in a less important tissue like the periosteum of a long bone, may produce no discernible clinical signs and symptoms at all. On the other hand a larger number of spirochetes scattered about in the heart muscle and walls of the larger blood vessels or elsewhere may produce relatively large amounts of "reagin" yielding strongly positive Wassermann reactions without necessarily being accompanied by a commensurate degree of physical disability and sickness. We think it is important for physicians to keep this possibility in mind as affording a reasonable explanation for the occurrence of truly positive Wassermann reactions with the sera of individuals without discoverable clinical evidences of syphilis.

If, therefore, we are correct in our surmise that the "reagin" or "alexofixagin"* in the blood and spinal fluid of cases of syphilis is produced by the lymphocytes and other cells in contact with *Spirochete pallida* and its products in the tissues then we must be prepared to admit that the reaction may be negative in such cases of syphilis in which the spirochetes are so latent that they exist in the tissues with little or no cellular reaction as indicated by the histologic studies of Warthin and his colleagues. We must also admit at the same time that our complement-fixation test must be as sensitive as consistent with specificity in order to detect as many such cases as possible and especially since they may quite easily escape clinical diagnosis. Their detection may not necessarily indicate the institution of active antisyphilitic treatment; this is a separate question not directly concerned in the present discussion, but they nevertheless require our best diagnostic efforts and especially when the complement-fixation reaction is being employed as an aid for determining cure in order to avoid the very regrettable error of undertreating the disease since such spirochetes may suddenly and unexpectedly spring into renewed activity at subsequent periods in the individual's life.

We believe therefore that even with the most sensitive methods there will always occur a certain unknown percentage of falsely negative Wassermann

*A term employed by one of us (J. A. K.) for designating the complement or alexin fixing type of antibody.

reactions in cases of extremely latent syphilis because of insufficient amounts of "reagin" in the blood. But it is the plain duty of serologists to employ methods as sensitive as consistent with specificity to reduce to a minimum the percentage of these falsely negative reactions. We are convinced that efforts of this kind require unremitting attention and a high degree of technical skill, but when one pauses to consider for a moment the potential importance of the majority of Wassermann tests to the individuals concerned, to members of their immediate family and to the state, it is readily realized that the complement-fixation test for syphilis is by all odds the most important routine procedure today in medical laboratory practice worthy of our best efforts and in the conduct of which mere economy of time and materials and the desire for the return of quick reports are certainly much less important than the accuracy of results. An error in urinalysis, a blood count, a bacteriologic examination, etc., does no particular harm and if it has made an impression upon the patient and his physician is readily corrected, but an error in the Wassermann reaction and especially the occurrence of a falsely positive reaction, may be even more serious than an erroneous diagnosis of cancer in a tissue leading to a dangerous operation.

In our efforts to render the complement-fixation test for syphilis as sensitive as consistent with specificity we may run into the danger of nonspecific falsely positive reactions, but these are avoidable by correct technical procedures. Likewise errors in technic may occur as long as the test is conducted by human hands although the incidence of these can be reduced almost to a negligible minimum by confining the conduct of the tests to responsible and skillful workers but the very important question not infrequently arises, do changes occur in the blood in nonsyphilitic diseases that may lead to falsely positive reactions with technically acceptable methods? In other words may an antibody-like substance occur in the blood in diseases other than syphilis capable of causing the flocculation of lipoids in colloidal suspension with the absorption or fixation of complement similar to the mechanism of the Wassermann reaction? This is a question of fundamental importance because it concerns the possibility of the occurrence of biological, and not technical, falsely positive reactions.

We think it is to be granted that in trypanosomiasis, leprosy and possibly in a few other febrile diseases that changes *may* occur in the colloidal chemistry of the blood which increase the tendency of the serum to yielding nonspecific reactions by a mechanism similar to that responsible for the occurrence of positive Wassermann reactions with the sera of healthy rabbits, dogs and mules. The mechanism of this kind of reaction is not known and it is hardly necessary at this time to attempt offering an explanation, but the phenomenon is not merely one of anticomplementary action of serum so readily detected in the serum control tube. It is rather of a nature similar to the proteotropic reaction of Noguchi which refers to falsely positive Wassermann reactions with unheated normal human serum due to the fixation of complement by some interaction between the serum and the proteins of alcoholic tissue extracts employed as antigens. In other words these falsely

positive reactions are avoidable by the use of superior antigens and such technical procedures as enhance the degree of specific complement fixation by syphilis "reagin" while depressing the degree of nonspecific fixation by serum alone, antigen alone or a combination of antigen and various constituents present in all sera apart from the syphilis reagin.

Experience with the new complement-fixation test for syphilis devised by one of us (J. A. K.) during the past three years and covering many thousands of reactions reported by investigators in different parts of the country have established that the new method when properly conducted possesses three outstanding properties, namely, (a) a very high degree of specific sensitiveness; (b) an almost total absence of tendency for yielding apparently nonspecific reactions and (c) a very low percentage of anticomplementary reactions. We believe these results are largely due to four factors, namely, (a) to the employment of a new antigen which permits the use of a dose of 10 antigenic units which amount is yet 30 to 50 times less than the anticomplementary unit; (b) to the employment of a period of primary incubation of 18 to 20 hours at 6 to 8° C. followed by 10 minutes at 38° C. which greatly favors the fixation of complement by syphilis "reagin" and this extract while minimizing the degree of fixation by serum alone and extract alone; (c) to the use of an antisheep hemolytic system which permits the use of small amounts of guinea pig serum complement and rabbit antisheep hemolytic serum and thereby reducing to a minimum the presence of various serum constituents capable of interfering with specific complement fixation and (d) to the use of varying amounts of patient's serum which in a small but appreciable percentage of cases increases the specific sensitiveness of the test. As previously stated it cannot be hoped nor is it expected, that the new method is proof against technical errors but we believe that when it is properly conducted with an acceptable antigen that the positive reactions in this country are due alone to syphilis since frambesia or yaws is practically unknown.

TRYPANOSOMIASIS

We have had no opportunity for applying the new test in human cases of trypanosomiasis but a very large series of tests with the sera of rats, guinea pigs and rabbits infected with *Tr. equiperdum* conducted with the assistance of Miss Rule have shown that positive reactions do not occur with the new antigen although with antigens of the trypanosome strongly positive reactions are generally observed as early as one week after infection. The results of one such experiment are shown in Table I as an example of the whole series.

LEPROSY

Probably most interest is to be placed in the possibility of nonspecific reactions in leprosy. Undoubtedly the serum in this disease and particularly from cases of well advanced nodular leprosy may yield positive Wassermann reactions with most methods in common use but in a study of the new complement-fixation test in this disease by Denney and one of us[1] it was found

TABLE I

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN EXPERIMENTAL TRYPANOSOMIASIS*

RABBIT	C. L. ANTIGEN:**				SERUM DOSES:			TRYPANOSOME ANTIGEN:			SERUM DOSES:			
	.025	.012	.006	.003	.0015	.0008	Serum Contro	025	.012	.006	.003	.0015	.0008	Serum Control
BEFORE INFECTION	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1 week	–	–	–	–	–	–	–	*****	3	1	–	–	–	–
2 weeks	–	–	–	–	–	–	–	4	4	4	4	4	2	–
3 weeks	–	–	–	–	–	–	–	4	4	4	4	4	4	–
4 weeks	–	–	–	–	–	–	–	4	4	4	2	1	–	–

*Infected with *T. equiperdum.*
**C. L. refers to the new Cholesterolized and Lecithinized Antigen.
***– = negative; 1 = +; 2 = ++; 3 = +++; 4 = ++++.

that falsely positive reactions did not occur. Positive reactions were, of course, observed, but in these there was either historical or clinical evidence of syphilis and we believe that the new test is not at all likely to yield non-specific positive reactions in pure leprosy due primarily to the fact that the dose of antigen employed is 30 to 50 times less than its anticomplementary unit. With the old three antigen method apparently falsely positive reactions occurred in at least 7 to 8 per cent of apparently nonsyphilitic leprous patients in confirmation of numerous similar reports in the literature but these were not observed with the new method (Table II).

TABLE II

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN LEPROSY*

CASES OF LEPROSY	NUMBER TESTED	NEW METHOD		OLD METHOD	
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
With no Evidences of Syphilis	125	None	100%	7.2%	92.8%
With Evidences of Syphilis	27	100%	None	100%	None
Syphilis Suspected	7	None	100%	None	100%

*159 Cases: Nodular, 39; anesthetic, 32; mixed, 88.

TUBERCULOSIS

Recently Kilduffe[2] has shown that the new antigen does not yield falsely positive reactions in tuberculosis of nonsyphilitic individuals and a large series of tests conducted by one of us (J. A. K.) in cooperation with Dr. Callahan and to be separately reported upon, have yielded similar results. We are sure that the sera of nonsyphilitic tuberculous subjects do not yield positive reactions although several investigators claim that this may occur with the sera of some patients tested with the ordinary cholesterolized extracts.

PREGNANCY

The same is true with the sera of nonsyphilitic pregnant women tested at or near term. In the experience of Smith[3] the new test does not yield falsely positive reactions in this condition and the experience of one of us (J. A. K.) has been exactly similar. When positive reactions have occurred they have remained positive after delivery and have been observed only in

syphilitic women. In this connection it is to be borne in mind that pregnancy may stimulate or provoke latent syphilis into activity just as trauma, intercurrent disease, the administration of arsphenamine, etc., may prove provocative so that unexpected positive reactions may occur but when these are observed with the new method we believe that syphilis is present rather than that the reactions are falsely positive due to metabolic, toxic or other changes in the serum in late pregnancy.

DIABETES MELLITUS

Recently Rockwood and Sanford[4] have shown in an excellent serologic study of 501 cases of diabetes that falsely positive Wassermann reactions are of rare occurrence in this disease. Among these there were eight cases of probable coincidental syphilis with apparently falsely negative reactions with the sera of two by a modified Noguchi method and one by the Kolmer method;

TABLE III

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC CASES OF DIABETES MELLITUS

NO.	SEX	AGE (YRS.)	BLOOD SUGAR (MG. PER 100 C.C.	URINE SUGAR (PER CENT)	ACETONURIA	LIPEMIA	QUANTITATIVE COMPLEMENT FIXATION REACTION
1	M	43	378	6	+++	0*	–*
2	F	24	170	2	0	Slight	–
3	M	60	246	2.5	+++	Marked	–
4	F	65	0	5	0	0	–
5	F	41	261	3.3	0	0	–
6	F	59	270	6	+	0	–
7	M	44	258	4.5	+	0	–
8	M	38	322	7	+++	Marked	–
9	F	56	0	4.2	0	0	–
10	F	58	300	5.6	0	0	–
11	F	60	0	2	0	0	–
12	M	50	374	7	++	Marked	–
13	F	57	0	3.5	0	0	–
14	M	60	272	3.3	+	Slight	–
15	F	32	332	7	++	Moderate	–
16	F	33	206	3	0	0	–
17	M	43	180	5-6	+++	–	–
18	M	68	308	6	++	Moderate	–
19	F	41	230	3-4	0	0	–
20	F	56	460**	4	0	0	–
21	F	29	236	5	+	–	–
22	F	42	240	4.5	0	Moderate	–
23	M	29	220	5	++	Marked	–
24	M	65	0	6	++	0	–
25	M	37	0	5.8	0	0	–
26	F	42	239	3.5	0	Moderate	–
27	F	32	172	2.4	0	0	–
28	M	37	186	3	–	0	–
29	F	70	0	4.5	–	0	–
30	F	47	178	1.5	–	0	–
31	M	43	210	4 8	++	Moderate	–
32	F	41	180	3	+	0	–
33	F	56	294	2	0	Marked	–
34	M	43	198	4.5	++	0	–
35	M	48	170	3	0	Moderate	–

*– = Negative; 0 = not determined.
** = Plasma CO_2 48 volume per cent.

five yielded positive reactions by the Kolmer technic but in two of these the evidences of syphilis were not conclusive. In 35 cases studied by us, several in coma, the reactions were negative as shown in Table III and it is our firm conviction that positive reactions by the new method in diabetes and especially repeated positive reactions are always due to coincident syphilitic infection.

MALARIA

Last year at the meeting of this society one of us[5] reported that in a study of the new test in malaria with sera kindly furnished by Dr. Bates of Ancon, Panama, and Dr. Caldwell of the Public Health Service in Houston, Texas, the reactions were uniformly negative with all but two negro patients

TABLE IV

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC CASES OF NEPHRITIS

NO.	SEX	AGE (YRS.)	ALBUMINURIA AND CASTS	TOTAL PHENOLSULPHONEPHTHALEIN RETURN IN 2 HOURS	BLOOD UREA (MG. PER 100 C.C.)	QUANTITATIVE COMPLEMENT FIXATION REACTION
1	F	34	+	5	145	–*
2	F	64	+++	10	52	–
3	F	45	+	35	25	–
4	F	19	+	0*	60	–
5	F	18	+	0	21	–
6	M	49	+	10	101	–
7	M	52	+++	0	67	–
8	M	61	+++	0	37	–
9	F	79	+	15	70	–
10	M	72	+	12	48	–
11	F	39	+++	65	15	–
12	M	42	+	0	150**	–
13	M	47	+++	0	55**	–
14	M	19	–	0	168***	–
15	M	51	++	0	179****	–
16	M	20	+	0	120	–
17	M	34	+	None	130*****	–

*0=not done; – =negative.
**=uremic coma.
***=uremic coma due to anuria.
****=blood creatinine 16.
*****=blood creatinine 4.

in whom the possibility of syphilis could not be definitely excluded. Since then tests with the sera of additional cases of tertian and aestivo-autumnal cases of malaria in individuals in whom the presence of syphilis could be excluded with a fair degree of accuracy, have yielded uniformly negative reactions and we are of the firm belief that the sera of nonsyphilitic malarial patients do not yield falsely positive reactions by this new method even when blood is drawn just before, during or after a paroxysm of chills and fever. The occurrence of positive reactions in malaria is presumptive evidence of syphilis requiring very careful clinical study.

NEPHRITIS, ICTERUS AND PNEUMONIA

From time to time investigators have reported that falsely positive Wassermann reactions may occur with the sera of nonsyphilitic individuals with

chronic nephritis with high retention, with icterus and pneumonia. In our experience reactions of this kind do not occur with the new method and we believe that they are relatively rare and exceptional with other methods in common use.

As shown in Table IV there is no tendency at all for falsely positive reactions in nephritis of nonsyphilitic individuals even when the blood is drawn during uremic coma. As shown in Table V falsely positive reactions do not occur with the sera of nonsyphilitic jaundiced individuals even when the sera are deeply colored with bile products which tends to increase their anticomplementary properties. The only case of croupous pneumonia in our series of cases which yielded a positive reaction was the serum of a syphilitic

TABLE V

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC CASES OF JAUNDICE

NO.	SEX	AGE (YRS.)	JAUNDICE DUE TO:	QUANTITATIVE COMPLEMENT FIXATION REACTION
1	F	46	Cholecystitis, etc.	–*
2	F	41	Cholecystitis, etc.	–
3	M	45	Cholecystitis, etc.	–
4	M	59	Carcinoma Pancreas	–
5	M	51	Carcinoma Pancreas	–
6	F	39	Cholelithiasis, etc.	–
7	F	50	Cholecystitis, etc.	–**
8	F	52	Biliary cirrhosis	–
9	M	62	Carcinoma Pancreas	–
10	M	38	Cholecystitis, etc.	–
11	F	47	Cholecystitis, etc.	–
12	F	38	Cholecystitis, etc.	–
13	M	50	Cholecystitis, etc.	–
14	F	50	Cholecystitis, etc.	–
15	F	43	Cholecystitis, etc.	–

*Negative.
**Serum discolored in dilutions as high as 1:40.

TABLE VI

COMPLEMENT-FIXATION REACTIONS BY THE NEW METHOD IN CLINICALLY NONSYPHILITIC CASES OF CROUPOUS PNEUMONIA

NO.	SEX	AGE (YRS.)	TEMPERATURE (°F)	DAYS BEFORE CRISIS	QUANTITATIVE COMPLEMENT-FIXATION REACTION
1	M	20	101	5	–*
2	M	17	102.4	2	–
3	F	28	100.6	1	–
4	M	31	101.2	2	–
5	F	40	101.4	1	–
6	M	26	100	During	–
7	F	38	102.4	2	–
8	F	42	103	1	–
9	M	61	101.4	3	–
10	M	59	102	2	–
11	M	45	101.8	5	–
12	M	62	102	2	–
13	M	33	101.4	1	–

*Negative.

with a Type IV infection (Table VI). We feel absolutely certain that the sera of nonsyphilitic individuals in advanced nephritis, in jaundiced and in croupous pneumonia before or directly after the crisis, do not yield falsely positive reactions in this method when it is properly conducted.

SCARLET FEVER

Finally, a few words may be written regarding the Wassermann reaction in scarlet fever. As reported to this society last year there is no evidence at all of the occurrence of falsely positive reactions in this disease by the new method and we believe the same may be said of most other complement-fixation methods in common use today. We believe that it may be stated without reserve that the serum in all stages of scarlet fever does not yield falsely positive Wassermann reactions. In any large series of cases unexpectedly positive reactions may be observed in from 1 to 3 per cent of cases as is true in a Wassermann survey of any large number of sick or healthy individuals but we believe that when these occur with technically acceptable methods that the individuals are worthy of very careful study from the standpoint of the presence of latent congenital or acquired syphilis or of clinically atypical active syphilis.

Errors in the complement-fixation test for syphilis may be divisible therefore into two kinds, namely, (a) those due to errors in technic in both a falsely positive and a falsely negative way and (b) those due to biological factors beyond the control of the serologist. The percentage of the first group may be reduced to an almost negligible minimum by careful technic with special reference to the qualities of the antigen and in this society devoted to the principle of preserving and increasing the accuracy and practical value of medical laboratory procedures it is to be hoped that the members, individually and collectively, will exert themselves to foster a proper appreciation of the tremendous importance which the complement-fixation test can be made to possess for the diagnosis of syphilis and as a guide in its treatment when conducted by properly qualified serologists unwilling to be hurried in their work or to adopt anything that sacrifices the maximum degree of sensitiveness consistent with the specificity of the reactions. Under these conditions we believe that the chances of securing falsely positive reactions will be practically negligible and to the best of our knowledge positive reactions in the absence of syphilis will occur only in frambesia, or yaws, which is almost unknown in the United States. Individually and collectively it should be our aim to render available to the clinician a complement-fixation test for syphilis in which the occurrence of positive reactions including the unexpected weakly positive reactions, shall demand, not an apology or excuse on the part of the serologist, but a very careful reinvestigation of the history and clinical status of the patient for evidences of syphilis on the part of the clinician.

CONCLUSIONS

1. Falsely negative complement-fixation reactions may occur in cases of extremely latent syphilis because of insufficient amounts of "reagin" in the

blood and spinal fluid. The incidence of these may be reduced to a minimum by employing a test yielding reactions of as maximum a degree of sensitiveness as is consistent with specificity.

2. Falsely positive reactions are usually due to errors in technic and can be reduced to a minimum by confirming the conduct of the complement-fixation test for syphilis to competent serologists unwilling to sacrifice accuracy for mere speed in returning reports or for economy of time and materials.

3. The new complement-fixation test does not yield falsely positive reactions with the sera of rats, guinea pigs and rabbits with acute and chronic trypanosomiasis.

4. The new complement-fixation test for syphilis does not appear to yield falsely positive reactions with the sera of *nonsyphilitic* lepers or with the sera of *nonsyphilitic* individuals with acute and chronic tuberculosis, acute and chronic malaria, in advanced pregnancy, advanced diabetes, advanced neph ritis, acute pneumonia, acute scarlet fever or with jaundice due to nonsyphilitic involvement of the liver and its bile ducts.

REFERENCES

[1]Kolmer, J. A., and Denney, O. E.: The Wassermann Reaction in Leprosy with Special Reference to the New Complement-Fixation Technic, Arch. Dermat. and Syph., 1923, viii, 63.
[2]Kilduffe, R. A.: Concerning the Cross-Fixation of Syphilitic Antigen by Tuberculous Sera, Jour. Lab. and Clin. Med., 1923, ix, 124.
[3]Smits, F. C.: The Syphilis Complement-Fixation Reaction in Pregnancy with Special Reference to the Kolmer Reaction, Amer. Jour. Syph., 1922, vi, 705.
[4]Rockwood, R., and Sanford, A. H.: The Wassermann Reaction in Diabetes, Amer. Jour. of Syph., 1923, vii, 679.
[5]Kolmer, J. A.: The Specificity and Sensitiveness of the Author's New Complement-Fixation Test for Syphilis, Jour. Lab. and Clin. Med., 1924, ix, 468.

SOME PHYSICOCHEMICAL ASPECTS OF HEMOLYSIS*

By Edward C. Mason, M.D., Detroit, Mich.,† and Reed Rockwood, M.D., Rochester, Minn.

INTRODUCTION

STUDIES in hemolysis have revealed the fact that salt solutions which prevent hemolysis, do so in concentrations having very nearly the same osmotic pressure; therefore, the theory has been advanced that the red corpuscle is enclosed in a semipermeable membrane, which swells or shrinks in response to the lowering or increasing of the osmotic pressure of the mediums surrounding the cell. Such a theory necessitates the assumption that the

*Read before the Third Annual Meeting of the American Society of Clinical Pathologists, June 5-7, 1924.

†The experimental part of this work was practically finished while at the Mayo Clinic, Rochester, Minnesota.

An abstract of this paper is to be included in the report of the annual meeting of the Physiological Society held at St. Louis, December 27-29, 1923.

hemoglobin is retained within the cell by a limiting membrane which, when taxed to the point of breaking, ruptures and allows the discharge of hemoglobin from the cell.

With more evidence at hand, one is inclined to question whether osmotic pressure plays any part in the process of hemolysis, for it is a well-established fact that isosmotic solutions of all salts and nonelectrolytes are not equally effective in preventing hemolysis. Neither can one explain the hemolysis by acids, alkalies, saponin, sodium oleate, bile salts, etc., on the basis of osmotic pressure changes, nor can the protective action of serum in the presence of various hemolytic agents be explained on a basis of altered osmotic pressure.

EXPERIMENTAL DATA

That the limiting surface of the red blood cell does not act as a retaining membrane is supported by observations such as are recorded in Fig. 1, a photograph of red cells which have been sectioned in various planes. Two hemolyzed "ghosts" are shown in the lower left corner for comparison. The photograph was taken ten hours after the cells were cut, and it will be ob-

Fig. 1.

served that the stroma still retained the hemoglobin. Rollet observed that the cells could be mechanically injured without releasing the hemoglobin from its combination with the stroma and this observation has since been confirmed by others. Our method for sectioning the cells has been to grind them with washed sand in physiologic salt solution and float the cells from the mixture.

The various reactions of the red corpuscle warrants the conclusion that it is, essentially, a mass of colloidal components; the chief colloids, from the standpoint of hemolysis, being the stroma and the hemoglobin. Apparently the stroma-hemoglobin union is of a physicochemical nature and, judging from all the available evidence, has the characteristics of certain absorption phenomena. It is possible to use vehicles, other than stroma, to combine with the hemoglobin and the resulting combination behaves, in many respects, like the red corpuscle.

COMBINATION OF HEMOGLOBIN AND KAOLIN

If a solution of hemoglobin be filtered, it will pass through the filter paper unchanged; also, if it be shaken with kaolin and again filtered, it will

again pass through the filter; however, if the kaolin be first treated with primary potassium phosphate and the hemoglobin solution be then added, subsequent filtration yields a water clear filtrate. Believing that such hemoglobin-kaolin combination was due to the two colloids carrying opposite charges, we attempted to learn at what hydrogen-ion concentration the union

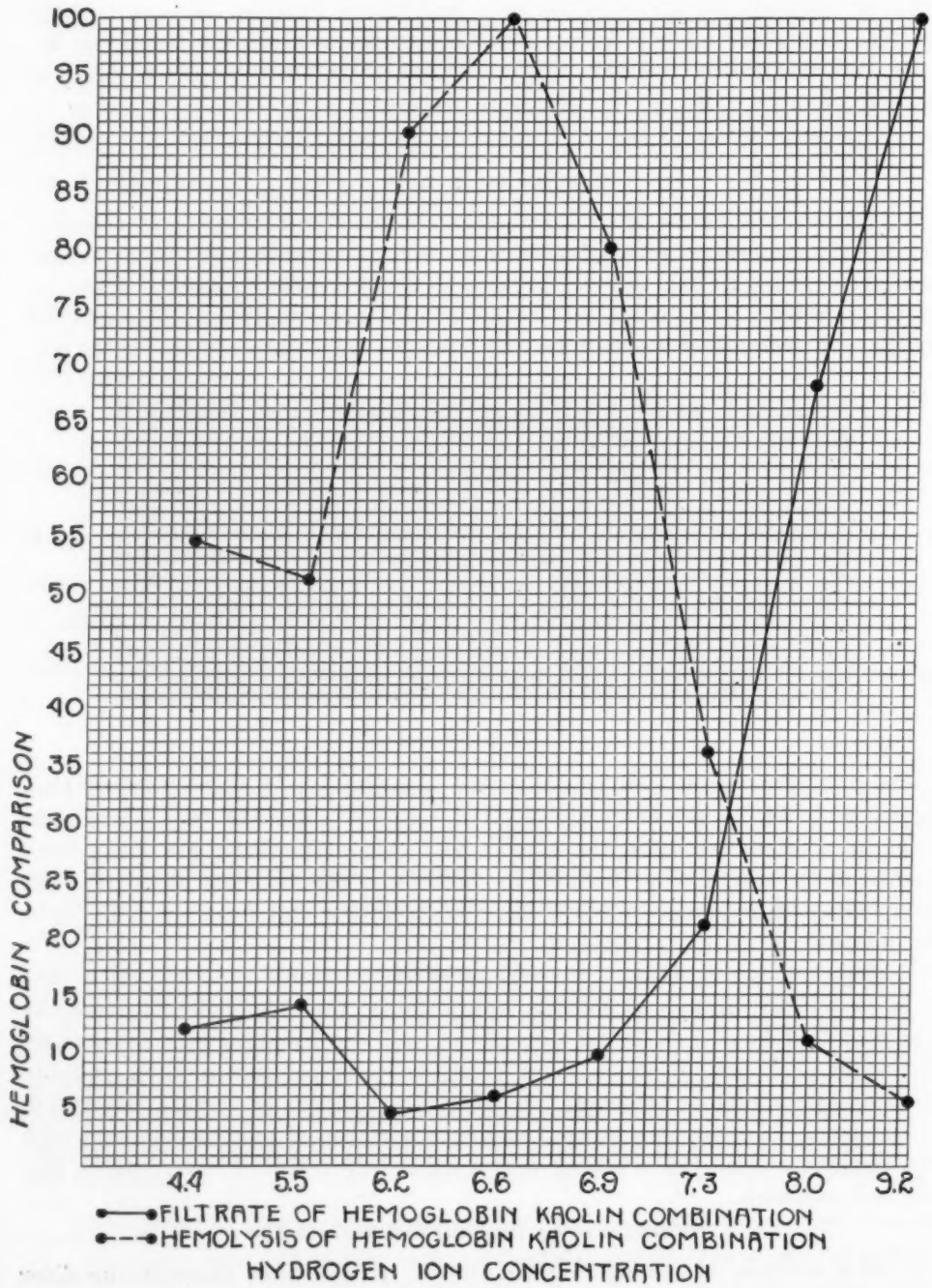


Fig. 2.

was most effective. Fig. 2 illustrates the degree of the hemoglobin-kaolin combination at different hydrogen-ion concentrations; eight tubes were used, each containing 5 c.c. of hemoglobin solution with 20 c.c. of phosphate buffer; 5 grams of kaolin were added to each tube and the whole well shaken. The tubes varied in hydrogen-ion concentration from pH 4.4 to pH 9.2. The filtrates from such hemoglobin-kaolin combination showed a marked difference in intensity of color. Intensity of color is expressed by the solid line

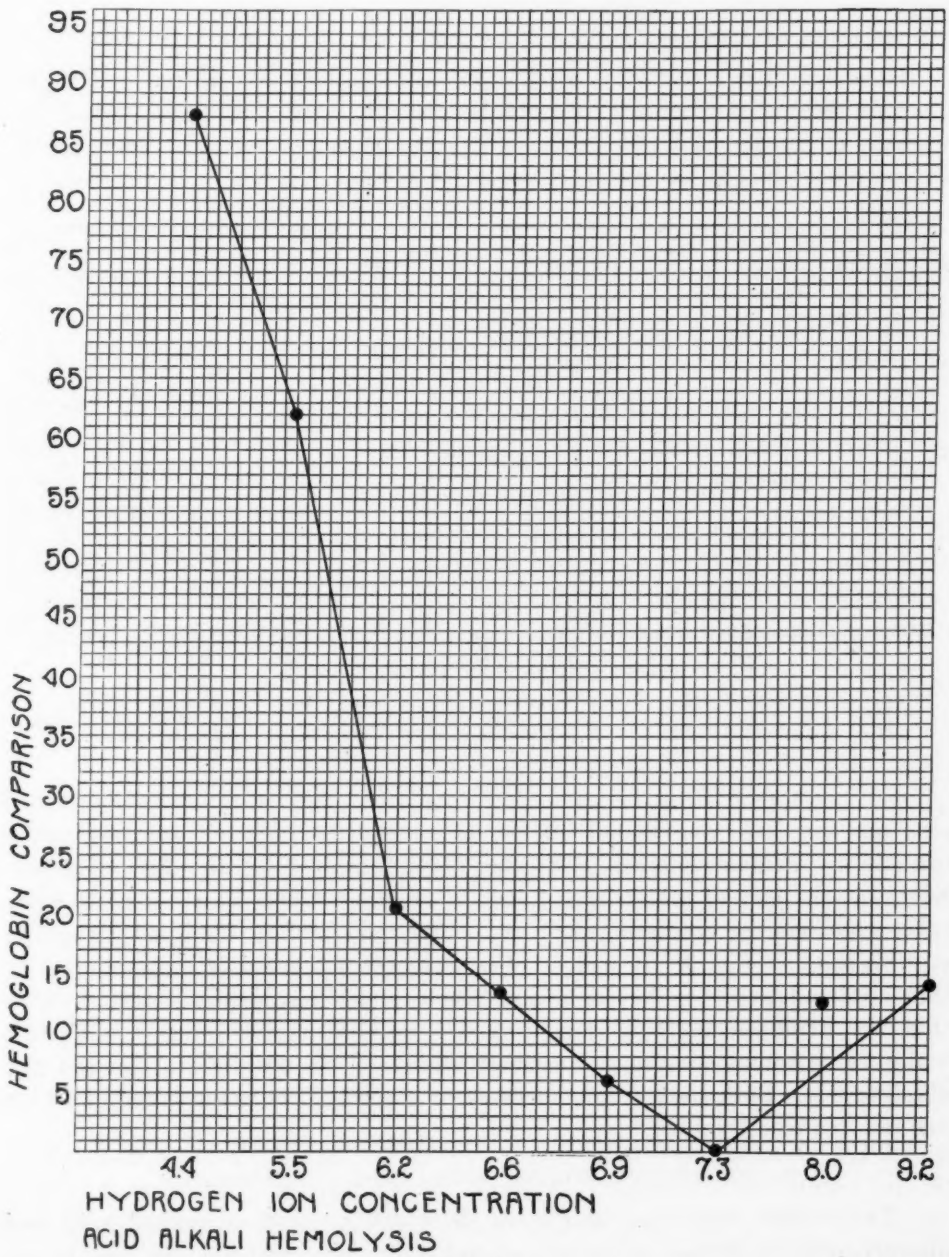
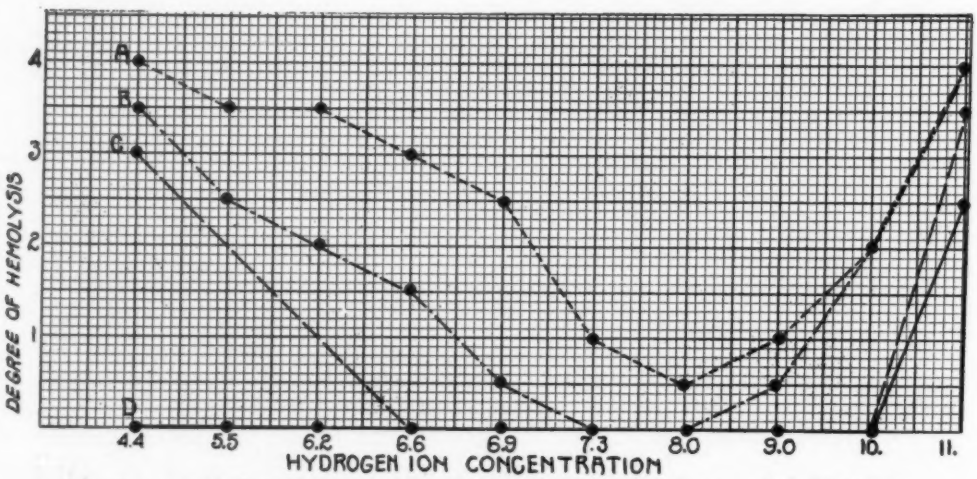


Fig. 3.

and is not absolute, but is the colorimeter reading obtained by using the least colored filtrate as the standard of comparison. The standard being practically colorless was given the value of 5. The precipitate remaining on the filter paper retained varying amounts of hemoglobin, the maximum, naturally, being in the one yielding the least colored filtrate. The precipitates were "hemolyzed" by treating each with an equal volume of concentrated solution of secondary sodium phosphate. The dotted line in Fig. 2 illustrates the results.

THE RELATION OF HYDROGEN-ION CONCENTRATION TO THE FRAGILITY OF RED CELLS

Believing that the stroma-hemoglobin combination of the red cell is, to a certain degree, similar to the kaolin-hemoglobin combination, we tested the response of the red cell to various hydrogen-ion concentrations. Our results are expressed in Fig. 3. Each of the eight tubes contained 1 c.c. of washed



A—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, 4.5 c.c. water, 32 hours.
B—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, 3.5 c.c. water, 20 hours.
C—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, 2 c.c. water, 17 hours.
D—1 c.c. washed cells, 10 c.c. isotonic phosphate buffer, after 5 hours.

Fig. 4.

cells and to each was added 10 c.c. of phosphate buffered solution made isotonic by sodium chloride. No hemolysis resulted in the first eighteen hours, at the end of which time 5 c.c. of distilled water was added to each tube, colorimetric readings were made and the results obtained gave the curve shown in Fig. 3. It will be observed that the curve is quite similar to that obtained from the kaolin-hemoglobin combination and differs mainly in that the kaolin-hemoglobin combination was most stable on the acid side, while the stroma-hemoglobin was most stable in the middle and toward the alkaline side. Both show a zone of greatest stability, the exact range of which has not been determined.

SALT PROTECTION AGAINST ACID AND ALKALI HEMOLYSIS

The results, expressed in Fig. 3, show salt protection against acid and alkali hemolysis, for, as previously stated, there was no hemolysis within the

first eighteen hours; however, when 5 c.c. of distilled water was added to each tube, there was definite hemolysis on both sides of the optimal hydrogen-ion concentration. Fig. 4 is presented to show somewhat the same condition but

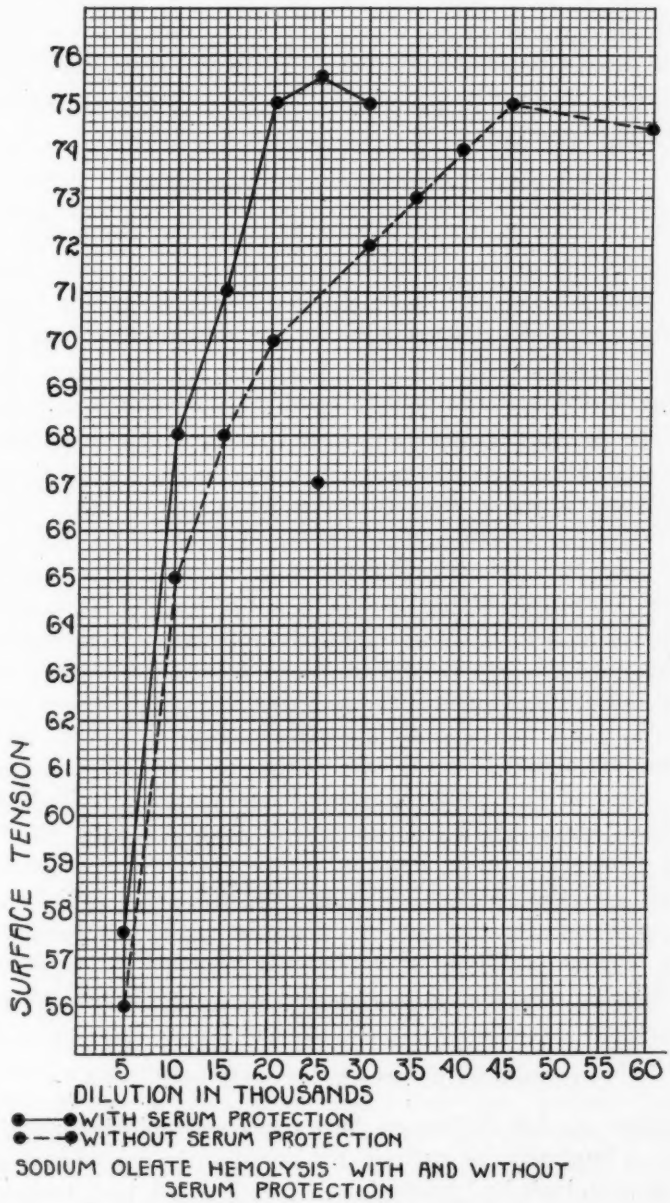


Fig. 5.

approached more gradually. Ten tubes were used, the range in pH being from 4.4 to 11 and the degree of hemolysis expressed in terms of 4, complete hemolysis being graded as 4. Each tube contained 1 c.c. of washed cells in 10 c.c. of isotonic phosphate buffer solution. Line D ex-

presses the results at the end of five hours, there being no hemolysis except in the tube with pH of 11. Water was gradually added, as expressed by C, B and A, and the resulting hemolysis charted. It will be observed that the tubes, more acid or more alkaline than the optimal range, became partially and even completely hemolyzed before any hemolysis appeared in the range of optimal hydrogen-ion concentration; also, since such hemolysis appeared only with dilution, it must be concluded that the salt concentration prevented acid and alkaline hemolysis.

SURFACE TENSION AND HEMOLYSIS

Many of the hemolytic agents have no chemical properties in common. However, lowering of surface tension is one striking physical property common to the most active. We have made determinations on surface tension and its relation to hemolysis, and an outline of our results is presented in Fig. 5. The apparatus used was that designed by DuNoüy.[2] The readings presented have not been corrected to read in dynes; they were recorded directly from the dial and should be accurate enough for comparison. The surface tension of sodium oleate was studied without and with the presence of serum protection. A series of twelve tubes was used, the first tube containing sodium oleate, in a concentration of 1-5000; the second, 1-10,000 and the dilution increased in each tube by 5000 parts of physiologic salt solution, until the last tube contained sodium oleate in a concentration of 1-60,000 of sodium chloride solution. It was observed that sodium oleate was active in producing hemolysis in concentration of 1-40,000 to 1-50,000. Surface tension determinations were made on the series of twelve tubes and found to range from 56 in the tube containing sodium oleate 1-5000 to 80 in the tube containing sodium oleate 1-60,000, the reading for salt solution being 91 (Fig. 5). It will be noted that, without serum protection, it required nine tubes to reach a surface tension of 75 and the dilution in such a tube was 1-45,000 but, with serum protection, only four tubes were needed to reach a surface tension of 75, and such a tube contained sodium oleate 1-20,000. These results are unexpected, for one would not anticipate that a substance, with as low surface tension as blood serum, would increase the surface tension of another substance. The results may be due to a direct combination of serum and sodium oleate.

HEMOLYSIS BY HYPOTONIC SALT SOLUTIONS

The surface tension difference, between a salt solution which is isotonic and one that is hypotonic, is entirely too slight to be considered as an important factor in hemolysis by hypotonic salt solutions. In hemolysis by hypotonic salt solutions, swelling preceded or accompanied the hemolysis and it is usually implied that, when swelling is sufficient to rupture the limiting "membrane," the hemoglobin is discharged. We have demonstrated that hemolysis may or may not be accompanied by increased volume of the red cell and, also, that cutting of the cell does not cause the discharge of hemoglobin.

DISCUSSION

We have prepared the model "red cells" which differ from those previously prepared in that we have used hemoglobin as one of the constituents. We have also been able to use various vehicles, other than stroma, to combine with the hemoglobin and have observed that such combinations were most effective over a zone near the neutral point.

Because of the lack of a better term to express the forces operating to combine these constituents, we have been compelled to use the much misused term "adsorption." Mathews has recently written a most illuminating paper on the subject of adsorption, in which he presents a plea for a less loose use of the term.

The following, under the section on "Chemical Adsorption of Substances and Solution by Solids," has a most direct bearing on the present discussion: "Under this heading are found most of those cases of adsorption with which we are familiar, such as decolorization by the use of charcoal; staining and dyeing; tanning; the precipitation of proteins; precipitation of one colloid by another; capillary analysis by means of filter paper. All of these are cases of adsorption, for the reason that the adsorbing substance is of a coarseness at least equal to that of the smallest colloidal particle in diameter. But this, however, does not alter their chemical powers, and in most or all of these adsorptions there can be little doubt that chemical substitutions rather than forces of surface tension, or physical forces, are involved."

It is evident that our kaolin-hemoglobin and stroma-hemoglobin systems belong to this comprehensive group; however, it seems impossible for us to rule out, as Mathews does, the influence of surface tension, for lowering of surface tension is one of the most effective methods of breaking the stroma-hemoglobin union.

We wish to agree with Mathews when he states that "surface tension is but one of the indirect results of the force of cohesion" and, also, that "cohesion is one of the forces of attraction of nature; surface tension is not." Therefore, since lowering of surface tension so closely parallels the breaking up of the stroma-hemoglobin adsorption, it appears wise to analyze the term "cohesion." Mathews states that "Chemical affinity is either electrical or magnetic, and probably the former; cohesion is neither of these. Chemical affinity appears to vary directly as the number of valences and, inversely, as the atomic weight, whereas, cohesion varied directly as the molecular weight, that is directly as the gravitational mass. Cohesion involves both gravitational affinity and chemical affinity." From the last sentence, it appears that cohesion is a much broader term than chemical affinity and, since chemical affinity does not comprehend surface tension changes, it becomes necessary for us to use the term cohesion. However, the term cohesion should be restricted to express attraction within a homogeneous system, and the term adhesion to express attraction within a heterogeneous system.

We have shown that the stability of the kaolin-hemoglobin and stroma-hemoglobin systems were disturbed with the addition of acid or alkali and have also shown salt protection against acid-alkali hemolysis. These facts,

taken together with the fact that the two amphoteric colloids, forming such systems, combine only within a zone close to the neutral point, suggests a possible relation to the observation made by Bredig. He observed that at the boundary of a particle in contact with water there is developed a force of surface tension. When the particle is electrically charged the surface tension is diminished, but, when the particle is discharged, the surface tension reaches a maximum. Therefore, at the isoelectric point, where the charge on the particle is nil, the surface tension is a maximal and such a point favors combination of the particles.

A study of blood coagulation and hemolysis furnishes some most interesting points of comparison. It will be observed that all agents, which tend to change the red cell from a state of (1) small mass, (2) low hydration capacity, (3) maximal cohesion and, (4) relative electrical neutrality, (all meaning essentially the same), to a state of (1) larger mass, (2) increased hydration capacity, (3) decreased cohesion and (4) away from the isoelectric point, do, to the same degree, promote hemolysis.

Coagulation of blood in some respects, on the other hand, appears to be just the reverse process of hemolysis. There is a rapid change, in blood coagulation, from a mass of high hydration capacity to one of low, and such process is accompanied by a marked change in cohesion, as shown by the enormous increase in surface tension. In regard to the isoelectric point during coagulation, it is interesting to note that the substances which accelerate coagulation are for the most part electronegative, while autolyzed tissue, which really represents tissue decomposed in relative acid condition, will retard coagulation.

CONCLUSIONS

1. The red blood cells may be cut without the subsequent discharge of hemoglobin.

2. Hemoglobin may be made to combine with vehicles other than stroma; such combination with kaolin is most effective over a definite zone of hydrogen-ion concentration.

3. The fragility of the stroma hemoglobin combination varies greatly with the hydrogen-ion concentration, the union being most stable in the zone of pH 7.3-8.0.

4. Charts are presented to show a definite salt protection against acid and alkali hemolysis.

5. Serum protection against hemolysis by sodium oleate parallels the increase of surface tension produced by adding the serum.

6. The stroma hemoglobin union is of a physicochemical nature and has the characteristics of adsorption phenomena.

REFERENCES

[1]DuNoüy, P. L.: A New Apparatus for Measuring Surface Tension. Jour. Gen. Physiol., 1919, i, 521-524.
[2]Mathews, A. P.: Adsorption. Physiol. Reviews, 1921, i, 553-557.
[3]Michaelis, L.: Die Wasserstoffionen-Konzentration, Berlin, 1914, pp. 49-50.
[4]Rollett, A.: Elektrische und thermische Einwirkungen auf das Blut und die Structure der rothen Blutkörperchen. Arch. f. Physiol., 1900, lxxxii, 199-267.

PHYSICOCHEMICAL ASPECTS OF HEMOLYSIS. II. AN ULTRAMICROSCOPIC STUDY OF HEMOLYSIS*

By Reed Rockwood, M.D., Rochester, Minnesota
(Fellow in Medicine, Mayo Foundation)

In connection with other studies[9] that have been made on hemolysis, the process of hemolysis was studied by means of the ultramicroscope, with special reference to the morphologic changes produced by different concentrations of different hemolytic agents.

Bechhold, Kraus, Salen and Hattori, working in Bechhold's laboratory, have reported observations on hemolysis with the use of the ultramicroscope. By the use of enzymes, various lipoid solvents, and protein precipitants, they have shown which portions of the cell are composed of protein, and which of lipoid, and they have demonstrated the various ultramicroscopic patterns formed when one or the other constituent is altered. Aside from this, their main interest has been in the effect of solutions of mercury, of which weak concentrations hemolyze the cell, and stronger concentrations precipitate the cell proteins.

As a result of these extensive observations, Bechhold believes that the hemolysis is due to changes in the stroma. Any condition which causes the stroma protein, cholesterin or lecithin to be separated will cause the release of the hemoglobin from combination. It is not necessary for all three constituents to be affected since, for example, a change in the lipoids alone may occur which will be effective in causing hemolysis.

METHOD OF STUDY

A Siedentopf cardioid condenser was used with a quartz object chamber. A 1000-watt Mazda lamp in a stereopticon served as the source of illumination. It was found that a water filter was inadequate to filter out the heat rays which would change to some extent the cell structure; a gold glass filter, which filters out 95 per cent of the heat rays was therefore substituted. Solutions of different percentages of the hemolytic agent were made, and one drop of solution was mixed in the chamber with one drop of freshly drawn human blood (taken from the same individual in each instance) and immediately examined.

In taking the photographs, a time exposure was found to be necessary. The Brownian movement in the cells often obscures the details of cell structure. For this reason a series of drawings has been added to show finer details. Because of the difficulty in keeping the illumination passing through

*From the Division of Medicine, Mayo Clinic.
Received for publication, May 6, 1924.
Read before the Third Annual Meeting of the American Society of Clinical Pathologists, Rochester, Minn., June 5-7, 1924.

the camera constant from day to day, the intensity of the light reflected from the cells appears to vary from photograph to photograph. The prints have therefore been retouched to bring out the proper contrast. In general, it may be said that there are only two degrees of intensity of light, one in the hemolyzed cell, and the other in the cell not hemolyzed. Certain exceptions to this occur in hemolysis by distilled water and in the reversal of hemolysis. The hemokonia, because of their small size and rapid motion, do not photograph. The difference in brightness between the hemolyzed "ghosts" and their background is very slight. It is very difficult to adjust the illumination so as to impress them on the plate. Therefore, the number seen in the photographs is usually considerably less than the number actually present. Photographs were taken of all the dilutions presented in this paper. Only a few are shown here as examples.

In the study of the cells a 1.8 mm. objective was used with a 20x Huygheneau eyepiece, giving a magnification of about 1900 diameters.

THE NORMAL ERYTHROCYTE

Normal human erythrocytes are well shown in Fig. 1. As seen from above, the corpuscle appears as a brightly illuminated ring surrounding a dark area, through which at times a little light is reflected from the lowest portion of the cell. This gives a false idea of the cell structure, and is due to the fact that the light is only reflected from the surface as it comes from the side. As the cells roll over and over in the field, however, or, as they are seen in rouleau formation, it is evident that all parts of the cell surface in turn will give a similar bright reflection.

THE TYPES OF ERYTHROCYTES SEEN IN HEMOLYSIS

Only a limited number of cell types are observed under different conditions (Figs. 2, 3, 4 and 5). Swollen forms, similar to the normal, but much enlarged, are sometimes seen (Fig. 2). The cell which has been hemolyzed appears as a faint gray-white ring standing out but slightly from the dark background, and smaller than the original cell from which it came (Fig. 3).

Certain cell forms have long been known to microscopists and called "crenated." It seems to me, after a careful study of these forms, that two groups have been included here: one, a true crenation, and the other, consisting of various degrees of stromatolysis. The truly crenated form is apparently a shriveling of the cell without cell destruction, and is probably due to some alteration in the water balance between the cell and its surrounding medium. The stromatolytic form is due to an actual destruction of the cell, particularly at its surface. Both forms may be combined to a greater or less extent, or a cell may first be crenated, and later stromatolysis occur. These forms may also be observed in ordinary microscopic fields from the same preparation, although their recognition is much facilitated by the intense illumination of the cell surface by the ultramicroscope. I cannot always distinguish between these different forms in the case of the individual cell, but by a study of several fields in the same preparation,

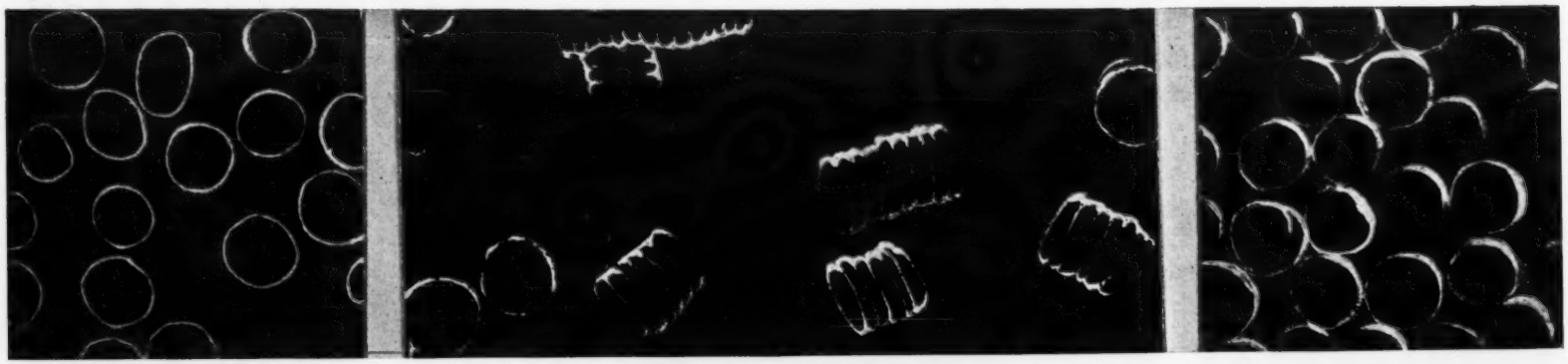


Fig. 1.—Normal erythrocytes.

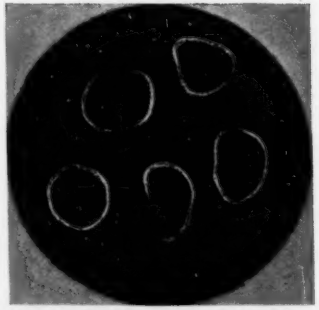


Fig. 2.—Swollen cells similar to the normal but much enlarged.

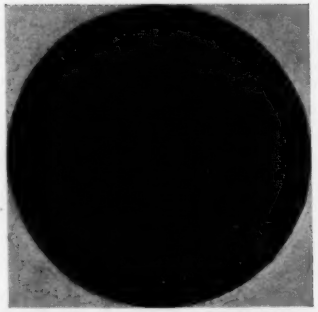


Fig. 3.—Hemolyzed cells.

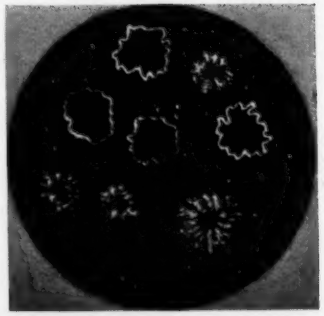


Fig. 4.—Crenated forms showing scalloping of the cell margin.

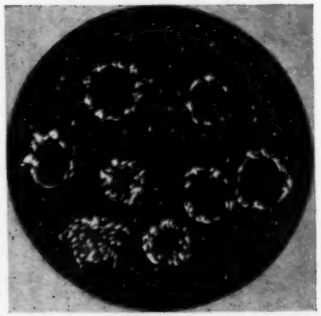


Fig. 5.—Stromatolytic forms, showing thickening of the outer rim with nodular distorted appearance.

observing cells as they roll over and over in the field, and watching the progress of hemolysis and stromatolysis, or by using different concentrations of the same reagent, I can usually decide, in a given preparation, which process predominates.

The crenation (Fig. 4) starts as a scalloping of the cell margin. By focusing it may be seen, however, that the surface of the cell is intact, and that the two borders of the ring of light surrounding the cell are nearly parallel, as in the normal cell. As the shriveling of the cell continues, small "chrysanthemum" and "hydrangea blossom" forms are produced, the original source of which is impossible to determine, unless the process has been followed, or unless distinguishing characteristics are present.

On the other hand, in the stromatolytic forms (Fig. 5), a thickening of the outer rim may produce a nodular distorted appearance. It suggests an aggregation of the colloids of the surface into larger particles. Then pieces of the cell surface may be distinctly seen to break off and float away into the solution, leaving gaps in the surface layer which persist even after careful focusing. This process may also result in chrysanthemum or hydrangea blossom forms, similar to those of the crenated cell. In certain cases the surface breaks down, forming long, trailing, gray-white streamers with brighter spots beaded along their course, giving a Medusa-like appearance. These forms are particularly observed in cells in which stromatolysis occurs after standing for a few hours at room temperature. Bechhold has noticed these forms as a result of treatment with mercury.

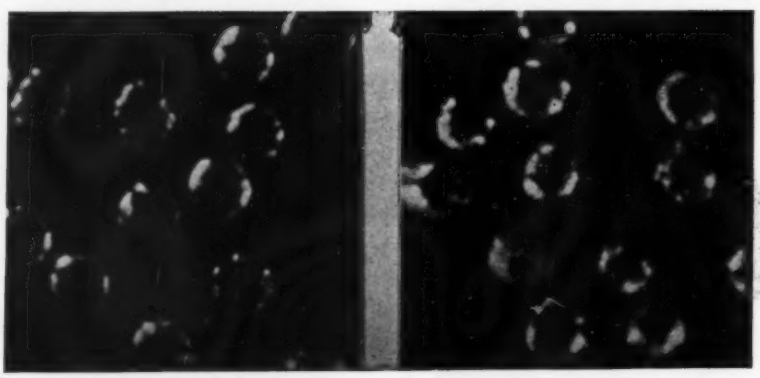


Fig. 6.—Stromatolysis without hemolysis.

HEMOKONIA-LIKE BODIES

Numerous brilliant granules, corresponding to the descriptions usually given for hemokonia, are seen in all of these preparations of the solutions of the hemolytic agent mixed with blood. The pieces of cell broken off in the process of stromatolysis cannot be distinguished with the microscope from those originally present in the serum. Porter, in making microscopic studies of autolyzing blood, also observed the formation of hemokonia-like bodies from these stromatolyzing forms. If the corpuscles are examined, diluted with serum in the chamber, their number is relatively small. If

diluted with istonic salt solution, there is a very distinct increase, and this also confirms the work of Hamburger, who found that cells which had been washed in isotonic salt solution or Ringer's solution revealed the presence of lecithin by chemical tests in the solution, and were less resistant to hemolysis. He found also that this lack of resistance could be done away with by the addition of more lecithin to the cells. The descriptions which have been given of hemokonia indicate a lecithin-like substance. These bodies are smaller and appear to be different from those described by Rous and Robertson as "fragmentation" forms. It should be noted that these particles are seen in shed blood after treatment with various reagents, and aside from their morphology there is nothing to identify them with the hemokonia seen in the circulating blood.

If other agents are added which cause distinct stromatolysis, the number of hemokonia-like bodies is increased enormously, and this increase appears to be roughly parallel to the amount of stromatolysis which is observed. It will be noted that there is an increase in the number of hemokonia-like bodies before any definite stromatolysis can be observed by the ultramicroscope, as in the case of isotonic salt solution.

CHANGES PRODUCED BY ALLOWING CELLS TO STAND

If corpuscles in oxalated blood are allowed to stand, even in the ice box, changes begin to occur relatively early. Within five or six hours distinct stromatolysis is evident, and numerous particles may be seen to be breaking off from the surface of the cells, even though they are standing in their original serum. Some crenation may be present as well, but the main process is that of stromatolysis. Hemolysis, under these conditions, however, does not occur for some days. Thus, the cells in the preparation illustrated in Fig. 6 show a marked stromatolysis of the cell surface, but hemolysis did not commence until two days after the photograph was taken. This cellular degeneration is not constant and occurs faster in some specimens of blood than in others. It seems to be favored by centrifuging, or by washing the cells in isotonic salt solution.

These observations show the relatively small part which the boundary between the cell and the plasma can play in hemolysis, since its marked alteration does not result with the release of the hemoglobin from the stroma. It has been shown[9] that the cell could be cut in pieces without the loss of hemoglobin. This boundary or apparent contact surface of the cell must be distinguished from the actual contact surface of the hemoglobin-stroma union and its surrounding intracellular medium.

HEMOLYSIS BY DISTILLED WATER

The actual process of hemolysis as seen under the ultramicroscope is substantially the same for all the hemolytic agents that have been studied. Two points are noticeable: the rather rapid destruction of the individual cell, which loses its hemoglobin in from three to five seconds, and the marked irregularity in the cell's resistance to hemolysis, many cells persisting for

a long time in the solution. This is particularly true with certain hemolytic agents. As the cell hemolyzes, it uniformly fades from the bright rim of light seen in the normal cell to the faint gray-white rim which outlines the disk of the ghost. At the same time it shrinks a little from its original size, and this process may continue for a short time after the rim has become completely pale.

In hemolysis by distilled water, there are one or two additional characteristics. The hemolysis is slower than the usual time of three to five seconds, and many intermediate gradations in brightness are visible between the primary and final forms. The cells are swollen and lose their biconcave appearance. There is no definite stromatolysis to be observed in the cells or ghosts, but there is a distinct increase in the number of hemokonia-like bodies in the field, particularly with the higher percentages of distilled water (60 to 70 per cent).

HEMOLYSIS BY ACID

In hemolysis by the addition of hydrochloric acid, there is also considerable agglutination which at times makes interpretation of the results difficult (Fig. 7). Hemolysis seems to depend not only on the concentration of the hemolytic agent, but also on the relative concentration of hemolytic agent and cells, and the time which is allowed to elapse. This is also characteristic of other hemolytic agents besides acid. Thus, with hydrochloric acid (N/500) with sodium chloride solution, hemolysis occurs in a few cells if not many are in the preparation, but not at all if there are quite a number in the field. From N/330 upward, agglutination begins to occur and this is more marked at about N/200. At N/166 and N/142, both hemolysis and agglutination are present. The agglutinated cells seem more resistant to hemolysis. With the higher concentration of acid, N/142, there also seems to be a little stromatolysis. This is independent of the hemolysis. That is, hemolysis may occur before stromatolysis or after, and the ghosts may be destroyed, in some cases after hemolysis.

HEMOLYSIS BY ALKALI

By the use of sodium hydroxid, nothing especially characteristic is seen. There is a little stromatolysis visible in about N/142 dilution and the cells hemolyze as described.

HEMOLYSIS BY SAPONIN

The hemolysis of individual cells in saponin (1/1000) is very rapid, but many cells are quite resistant. Most of the cells in 30 and 40 per cent dilutions of 1/1000 saponin hemolyze without any visible change in the cell surface, but the surface is almost immediately attacked, although at a slower rate, and glittering particles can be seen to become detached, giving typical hydrangea-blossom forms. These cells may or may not become hemolyzed. Four forms may be seen in the field at the same time in these concentrations: normal cells, normal ghosts, stromatolyzed cells, and stromatolyzed ghosts. The study shows the independence of the processes of hemolysis and stromatolysis.

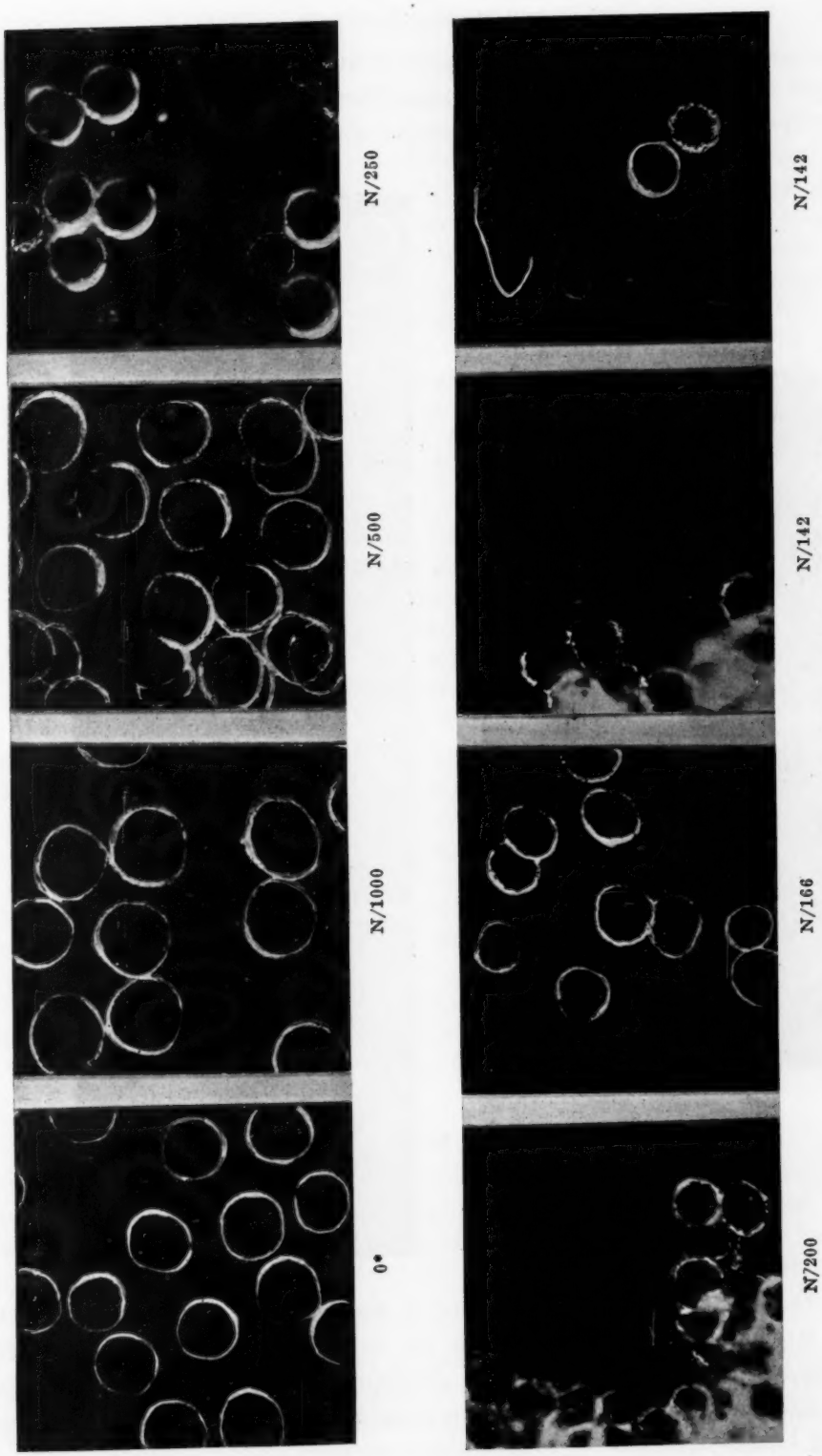


Fig. 7.—Hemolysis by acid. *Strength of hydrochloric acid.

HEMOLYSIS BY SODIUM OLEATE (1/1000)

Stromatolyzed forms are found very early in the series, but all seem to begin as crenation, although, later, particles can be distinctly seen to break off. There is a marked difference in the size of the cells, which seems to be

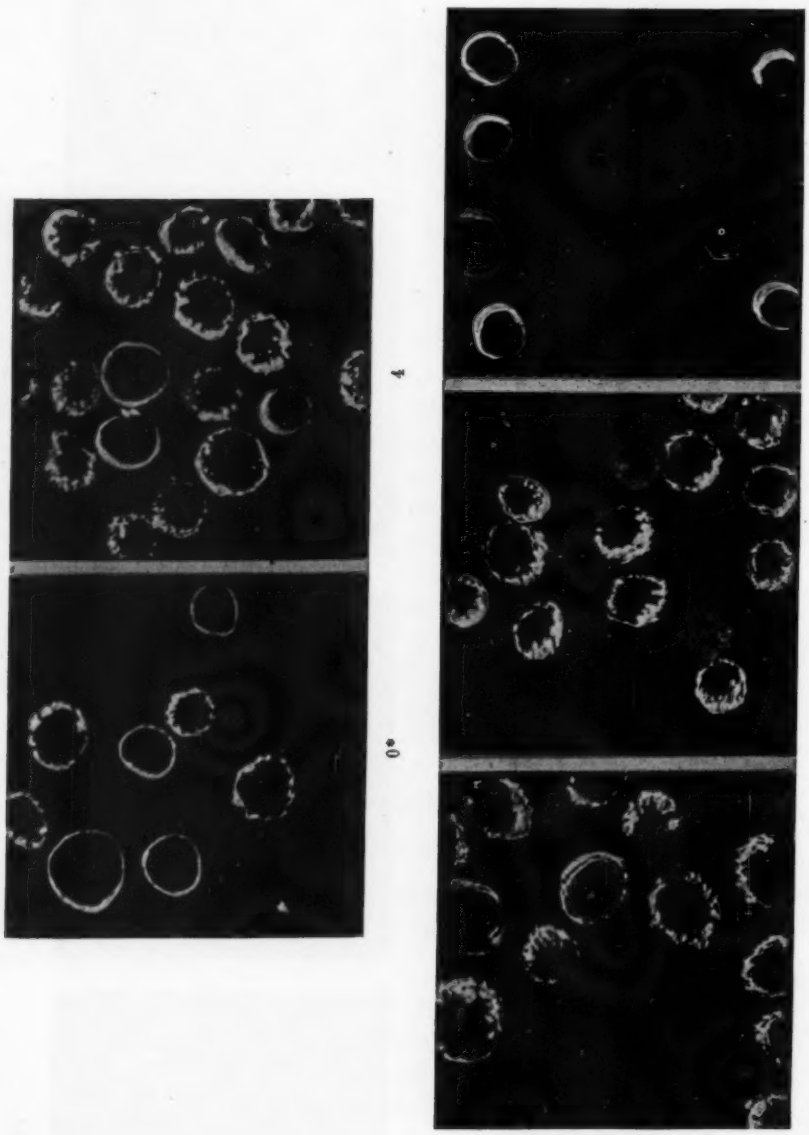


Fig. 8.—Hemolysis by autolysis and action of sodium chloride. *Percentage of 20 per cent sodium chloride.

due to swelling, and to a difference in resistance. Since normal, crenated, and stromatolyzed forms are seen in the same field, the latter predominating, it can be clearly demonstrated that stromatolysis and crenation are not essential to hemolysis, and that the processes go along independently. In most cases hemolysis seems to occur without swelling of the individual cell.

THE ACTION OF HYPERTONIC SALT SOLUTION

The action of hypertonic salt solution shows many curious phenomena which I am not able to explain. Crenation is greatest at from 2 to 6 per cent; there is some hemolysis at 6 per cent. Beyond this, crenation is slight and a large portion of the cells may even be swelled. Hemolysis also depends on the time of action since it becomes very marked in the 6 per cent solution if it is allowed to stand a number of minutes. Stromatolysis is also very definite at this concentration, and granules can be seen to break off singly and in groups. At 14 per cent there is a little crenation, although it appears in typical form in only a few cells. Hemolysis occurs, with and without crenation and stromatolysis, although principally without. The majority of the cells are not swollen, although a few in each field are markedly so. Distinct caput Medusa-like forms with waving arms, which may break off, are seen. These have been described by Bechhold, and I believe that they are merely atypical forms of stromatolysis.

HEMOLYSIS BY AUTOLYSIS AND HYPERTONIC SALT SOLUTION

By autolysis I mean the hemolysis and associated changes which take place in the red cell after standing from twelve to eighteen hours at room temperature. Simple autolysis causes extreme stromatolysis of the cell. Bacteria are seen at times. The hemolyzed stromas appear as usual, except that some may have brightly refractile granules still attached. Bechhold believes that these are inside the ghost, rather than on the surface. The granules throughout appear larger than those ordinarily seen. There is a tendency for a considerable number of the cells to appear swollen, but many are not. Some of the swollen cells show stromatolysis.

Autolysis may be prevented by the addition of from 2 to 4 to 8 per cent of hypertonic salt solution. If a higher concentration is used, hemolysis occurs again, probably due in part to the hemolytic action of the larger amounts of salt (Fig. 8). The mechanism of this action will be discussed in a later paper. In the high concentration of sodium chloride (10, 12, or 14 per cent), hemolysis has evidently occurred so rapidly that little time has been given for stromatolysis, and the cells have been protected in some manner so that stromatolysis does not occur to any extent, even after standing so many hours.

BIOLOGIC HEMOLYSIS

There is no definite morphologic change in the cells in hemolysis by complement and amboceptor. The surrounding rim fades out without any change in cell volume. Apparently this type of hemolysis releases the hemoglobin with less distinct disturbance of the cell than any of the others studied.

HEMOLYSIS BY HEAT

In order to observe the effect of heat on the red blood cell, the gold glass filter is removed from the source of illumination for one hour. Within a few minutes, a few cells hemolyze, and the number gradually increases. There is some stromatolysis, but this is relatively slight. These findings serve

as a control of our other observations, since in these cases the filter was kept in place and the observation of a single preparation was not extended over a few minutes.

THE REVERSIBILITY OF HEMOLYSIS

Many chemical and adsorption compounds form a more or less reversible combination. The question arises as to whether or not hemoglobin and

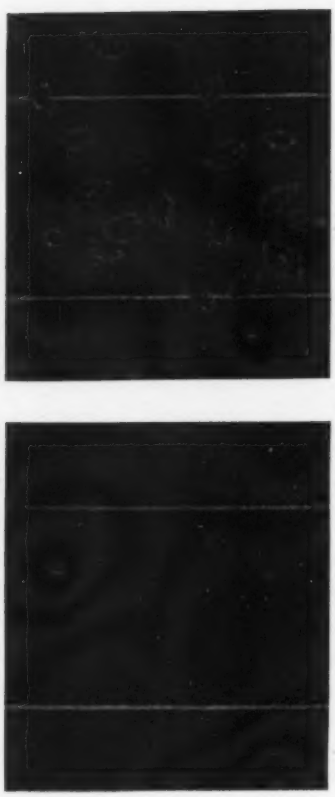


Fig. 9.—Hemolysis reversed by potassium chloride.

Fig. 10.—The reversal of hemolysis.

stroma form a similar combination. Relatively little work has been done along this line. One of the chief difficulties in demonstrating such reversal has been the failure to differentiate hemolysis from stromatolysis. Many hemolytic agents not only release the hemoglobin from combination, but also

injure the stroma, and thus the conditions necessary for reversion are no longer present. The presence of the cell "membrane" or phase boundary, altered though it may be, may also be a factor in preventing the return of the hemoglobin.

Koeppe, many years ago, noted that when red blood cells were centrifuged with 2 to 3 per cent ammonium nitrate, or at a very high speed, the laked blood lost its transparency, and it became opaque again. He did not recognize this as a reversal of hemolysis, however, and offered a complex explanation along other lines. Later, Spiro and Schwalbe hemolyzed cells with distilled water and showed that the hemolysis could be reversed by the addition of 5 per cent potassium chloride. Adair, Barcroft, and Bock observed reversal by salt, but ascribed it to an optical effect due to the swelling of the corpuscles.

I have confirmed this older work. The cells should be washed in isotonic salt solution, centrifuged, and then hemolyzed by adding distilled water. The solution becomes transparent. If 5 per cent potassium chloride

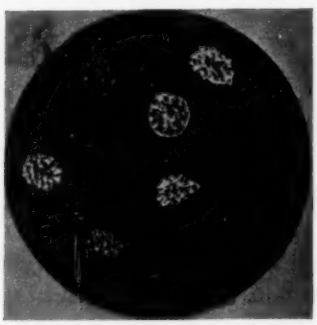


Fig. 11.—Reappearance of the crenated form of the erythrocyte.

is added, the solution becomes opaque again. Under the microscope in the hemolyzed solution no "ghosts" can be seen. After the potassium chloride has been added, however, the cells are seen to be numerous in the field (Fig. 9). Under these circumstances the reversal of hemolysis is never complete. The ultramicroscopic study of the same process shows that it is the crenated form of the erythrocyte which reappears (Figs. 10 and 11). The individual cells take up hemoglobin to a different extent. Some resume their original brightness, but there are all gradations between the restored crenated cell and the pale ghost. As a whole, the reversed cells are a little smaller than the stromas, but, contrary to the idea of Adair, Barcroft and Bock, the stromas are not swelled, but are rather a little smaller than the normal cell, so that an optical illusion due to swelling can be excluded.

The same result occurs with 5 per cent solutions of potassium chloride, sodium chloride, barium chloride, ammonium nitrate, potassium carbonate, acid potassium phosphate, and aluminum potassium sulphate, so that the result is apparently due to an alteration in the tonicity of the solution, rather than to any specific ionic effect.

DISCUSSION

At the last International Physiological Congress at Edinburgh, Brinkman reported further results on the reversal of hemolysis. Through the kindness of Brinkman and Szent Györgyi, I have received a copy of their paper in advance of publication. They, too, have repeated this older work and have extended it to include hemolysis by freezing and thawing, and hemolysis by linolenic acid. With this latter substance they have secured their most complete reversal. They have made careful studies of the refractive index of hemolyzed and reversed blood, and have clearly shown that in the course of hemolysis, all the hemoglobin leaves the cell, while with reversion it comes back to an extent of not less than 60 per cent. This definitely proves the actual fact of reversion and disposes of arguments based on optical effects.

I have also confirmed this work with linolenic acid. Under the ultramicroscope it can be seen that the cells are restored without crenation, so that they cannot be distinguished from the normal cells as shown in Fig. 1. Here again, there is a difference in the brightness of the various cells, due to incomplete reversal. Every cell seems to brighten in its outline, indicating that it takes up some hemoglobin, but certain cells resume the original degree of brilliancy. No stromatolysis is observed at any time in the stroma. No definite change in volume is observed during hemolysis by linolenic acid and reversion. Hemolysis by this substance approaches more closely morphologically that by complement and amboceptor than any which I have studied.

Other facts, also, point to the existence of reversed hemolysis. Thus, von Krogh has shown that hemolysis by sodium hydroxide can be reversed in part by the addition of the proper amount of hydrochloric acid. Fischer has made model erythrocytes of fibrin and carmin, and has shown that such mixtures are "hemolyzed" by distilled water, acid, alkali, urea, ammonia salts, autolysis, and heat, in a manner similar to the hemolysis of the true cell. Mason and Rockwood have shown in a previous paper that hemoglobin and kaolin, and hemoglobin and agar form reversible compounds which behave, in some respects, as do the true erythrocytes.

CONCLUSIONS

1. The ultramicroscope shows that many forms of cells which are commonly called "crenated" are, in reality, various forms of stromatolysis.

2. Stromatolysis and hemolysis are apparently independent processes, and stromatolysis is more marked with certain hemolytic agents at certain concentrations than at others.

3. The process of the formation of hemokonia-like bodies can be observed.

4. Hypertonic salt solution causes crenation in low dilutions, but causes stromatolysis, cell swelling and hemolysis as the concentration is increased.

5. Hemolysis by autolysis may be prevented by certain concentrations of hypertonic salt solutions.

6. The apparent cell surface plays a relatively small part in the process of hemolysis since it may be profoundly altered by stromatolysis at least two days before hemolysis occurs.

7. The fact that a true reversal of hemolysis may occur under the proper conditions may be confirmed by the ultramicroscope.

BIBLIOGRAPHY

[1]Adair, G. S., Barcroft, J., and Bock, A. V.: The Identity of Hemoglobin in Human Beings, Jour. Physiol., 1921, lv, 332-338.

[2]Bechhold, H.: Bau der roten Blutkörperchen und Hämolyse. München. med. Wchnschr., 1921, lxviii, 127-130.

[3]Bechhold, H., and Kraus, W.: Kolloidstudien über den Bau der roten Blutkörperchen und über Hämolyse, Biochem. Ztschr., 1920, cix, 226-235.

[4]Brinkman, H., and von Szent Györgyi, A.: The Reversion of Hemolysis, Jour. Physiol., 1923, lviii, 204-208.

[5]Fischer, M. H.: Edema and Nephritis, 2 ed., New York, Wiley, 1915, p. 364.

[6]Hamburger, H. J.: The Increasing Significance of Permeability; Problems for the Biological and Medical Sciences, Bull. Johns Hopkins Hosp., 1923, xxxiv, 266-274.

[7]Hattori, K.: Kolloidstudien über den Bau der roten Blutkörperchen und über Hämolyse. III. Ultramikroskopische Untersuchungen an Lipoide. Biochem. Ztschr., 1921, cxix, 45-64.

[8]Koeppe, H.: Über das Lackfarbenwerden der roten Blutscheiben. Dritte Mitteilung. Arch. f. d. ges. Physiol., 1905, cvii, 183-186.

[9]Mason, E. C., and Rockwood, R.: Some Physico-Chemical Aspects of Hemolysis, Jour. Lab. and Clin. Med., 1924, x, p. 10.

[10]Porter, F.: Observations on Blood Films, with Special Reference to the Presence of Hemoconia, Brit. Med. Jour., 1907, ii, 1773-1774.

[11]Rous, P., and Robertson, O. H.: The Normal Fate of Erythrocytes, Jour. Exper. Med., 1917, xxv, 651-673.

[12]Salén, E.: Kolloidstudien über den Bau der roten Blutkörperchen und über Hämolyse. II. Ultramikroskopische Untersuchungen an Stromata, Biochem. Ztschr., 1920, cx, 176-180.

[13]Schwalbe: Quoted by Ruszynák, S.: Beiträge zu einer Chemie der Hämolyse, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1911, viii, 421-432.

[14]Siedentopf, H.: Über einen neuen Fortschritt in der Ultramikroscopie, Ber. d. deutsch. physik. Gesellsch., 1910, viii, 6-47.

[15]Spire: Quoted by Ruszynák: Beiträge zu einer Chemie der Hämolyse, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig., 1911, viii, 421-432.

[16]von Krogh, M. L.: Ueber die Reversibilität der Hämolyse, Biochem. Ztschr., 1909, xxii, 345-351.

THE IMPORTANCE OF ABSORBING NATURAL ANTISHEEP AMBOCEPTOR FROM SERUMS TO BE USED IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By Ruth Gilbert, A.M., M.D., and M. J. Wemple, A.B.

THERE is considerable divergence in the results obtained by different investigators as regards the influence of natural antisheep amboceptor in human serums upon the complement-fixation test. Some workers find that the effect of natural amboceptor is so slight that it can be considered negligible while others believe that it introduces a definite source of error.

*From the Division of Laboratories and Research, New York State Department of Health, Albany, Augustus Wadsworth, M.D., Director.

Presented at the Meeting of the New York State Association of Public Health Laboratories, Albany, November 8, 1923.

Received for publication, March 6, 1924.

Those who hold the latter view have sought to eliminate this source of error either by utilizing the natural amboceptor in each serum or by absorbing it with sheep cells. The question of the practical necessity of removing natural amboceptor in this way has recently been studied in this laboratory.

To begin with, a satisfactory method, partly adopted from methods described by others, for absorbing natural amboceptor from human serums was developed. It is effective in removing amboceptor, does not cause the serum to become anticomplementary and is less time-consuming than some of the methods used in other laboratories. Briefly, it is carried out as follows:

To 1 c.c. of serum and blood cells (that is, blood poured from the clot before centrifugalization) 0.08 c.c. of washed packed sheep cells are added and the mixture is allowed to stand ten minutes at 3-6° C. The sheep cells and blood must be cold before mixing in order to prevent hemolysis due to the complement in the patient's blood. The serum recovered after centrifugalization is inactivated and is then ready for use in the complement-fixation test.

In a series of over 6000 serums submitted for the routine complement-fixation test for syphilis at this laboratory, only 730, or approximately 12 per

SUMMARY OF RESULTS OBTAINED IN COMPLEMENT-FIXATION TESTS BEFORE AND AFTER ABSORPTION OF NATURAL ANTISHEEP AMBOCEPTOR

Part I Readings Made Immediately After Hemolysis of Serum and Antigen Controls.
Part II Readings Made After the Tests Had Stood Overnight in the Cold Room.

	NUMBER OF REACTING SERUMS TESTED BEFORE AND AFTER ABSORPTION	NUMBER OF SERUMS SHOWING NO CHANGE IN DEGREE OF FIXATION		SERUMS SHOWING INCREASED DEGREE OF FIXATION AFTER ABSORPTION							
				TOTAL NUMBER OF SERUMS		NO FIXATION TO SLIGHT FIXATION		PARTIAL TO COMPLETE FIXATION		SLIGHTLY INCREASED DEGREE OF FIXATION	
		NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Part I	148	124	83.8	24	16.2	12	50.0	2	8.3	10	41.7
Part II	119	81	68.1	38	31.9	20	52.6	6	15.8	12	31.6

cent, were found to contain appreciable amounts of natural amboceptor. A specimen was considered to have an appreciable amount if the largest quantity of inactivated serum used in the complement-fixation test (0.04 c.c.) caused complete or nearly complete hemolysis of 5 per cent cells in fifteen minutes in the water-bath at 37° C. in the presence of two units of complement. The percentage of serums found to contain natural amboceptor in this series is from about one-fifth to one-half as high as that reported by other workers. This is probably due to the use of the shorter incubation period (fifteen minutes instead of sixty minutes at 37° C.) and to the use of only two units of complement (0.005 c.c. to 0.007 c.c. of guinea pig serum) instead of the fixed quantity 0.1 c.c. of a 1:10 dilution. It is also probable that the degree of hemolysis considered to represent appreciable amounts of natural amboceptor has varied with the different workers.

Of all the serums under consideration, 359 were used in the complement-fixation test before and after absorbing the natural amboceptor. Of these,

only 148 reacted at all in the tests read immediately after the hemolysis of controls so that our results (a summary of which is given in the accompanying table) are based upon these 148. Of these 148 reacting serums, 124, or 83.8 per cent, gave exactly the same results before and after absorption of the natural amboceptor. Twenty-four, or 16.2 per cent, gave greater fixation or fixation only after absorption. Of these 24 serums showing an increased degree of fixation after absorption, 12, or 50 per cent, showed a change from no fixation to slight fixation, 2, or 8.3 per cent, showed a change from partial to complete fixation and 10, or 41.7 per cent, showed a slightly increased degree of fixation. But taking 359, the total number of specimens tested before and after absorption (this includes the 212 giving no fixation as well as the 148 giving some fixation), the percentage giving greater fixation after absorption would be 6.6. From the fact that only 12 per cent of all routine serums examined were found to contain appreciable amounts of natural amboceptor, it could be estimated that less than 0.8 per cent would show any difference in reaction because of the removal of natural amboceptor.

When readings of complement-fixation tests were made after they had stood overnight in the cold room, the effect of the removal of natural amboceptor upon the reactions was very much greater than when readings were made immediately after hemolysis of controls. A summary of the results obtained with 119 reacting serums is given in this table. Thirty-eight serums, or 31.9 per cent, gave stronger fixation after absorption than before. Of these 38, 20, or 52.6 per cent, showed a change from no fixation to slight fixation, 6, or 15.8 per cent, showed a change from partial to complete fixation and 12, or 31.6 per cent, showed a slightly increased degree of fixation.

To summarize, our results have agreed with those reported by Olmstead and Kolmer in showing the effect of natural amboceptor to be less apparent if complement-fixation tests are read immediately after the secondary incubation than if read after standing overnight. The natural amboceptor has not been found to affect the complement-fixation reactions to any appreciable degree when readings are made immediately after the hemolysis of serum and antigen controls—so that when this method is followed, the absorption of natural amboceptor does not appear to be essential to the accuracy of the test.

BIBLIOGRAPHY*

Bauer, J.: Zur Methodik des serologischen Luesnachweises. Deutsch. med. Wchnschr., 1908, xxxiv, 698.

Hecht, Hugo: Eine Vereinfachung der Komplementbindungsreaktion bei Syphilis, Wien. klin. Wchnschr, 1909, xxii, 338.

Gradwohl, R. B. H.: The Hecht-Weinberg Reaction as a Control over the Wassermann Reaction, Jour. Am. Med. Assn., 1914, lxiii, 240.

Gradwohl, R. B. H.: The Hecht-Weinberg-Gradwohl Test in the Diagnosis of Syphilis, Jour. Am. Med. Assn., 1917, lxviii, 514.

Kaliski, D.: Specificity of the Noguchi Modification of the Wassermann Reaction, Arch. Int. Med., 1910, vi, 205.

Seelman, J. J.: Simple Method of Measuring Antisheep Amboceptor Content of Human Serum and Correcting for it in Wassermann Tests, Jour. Lab. and Clin. Med., 1917-18, iii, 626.

*No references to this brief bibliography are given in the text, as a discussion of the work of individual investigators would lengthen the paper unduly.

Stiner, O.: Ueber die Modifikationen der Wassermann'schen Reaktion nach Mintz und Rossi, Ztschr. f. Immunitätsf. u. exper. Therap., xviii, 378.

Rossi, O.: Ueber die Methodik der Wassermann'schen Syphilisreaktion. Ein Verfahren zwecks Absorption der im Menschenserum normalerweise enthaltenen Amboceptoren gegen rote Hammelblutkörperchen. Ztschr. f. Immunitätsf. Orig., 1911, v, 321.

Kahn, R. L.: What the Michigan Department of Health is Doing to Insure Correct Wassermann Results, Public Health, December, 1920, 486.

Ottenberg, R., and Frazier, B.: The Technic of the Wassermann Reaction with Reference to Thomas and Ivy's Method of Complement Dosage and to the Management of Natural Antisheep Amboceptor, Jour. Infect. Dis., 1915, xvi, 119.

Kolmer, J. A., and Rule, A.: The Influence of Natural Antisheep Hemolysin in Human Serum upon the Wassermann Reaction, Am. Jour. Syph., 1920, iv, 135.

Van Saun, A. I.: The Effect of the Natural Antisheep Hemolysin Content of Human Serum on Complement-Fixation Tests, JOUR. LAB. AND CLIN. MED., 1917-18, iii, 59.

Olmstead, M. P.: The Value of Absorption Methods in the Wassermann Test, Med. Rec., 1914, lxxxv, 341.

THE TREATMENT OF RHEUMATIC FEVER WITH THE ETHYL ESTER OF PHENYLCINCHONINIC ACID*

BY C. PHILIP MILLER, M.D., AND RALPH H. BOOTS, M.D., NEW YORK CITY

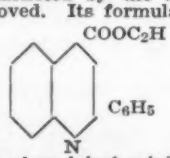
IN A previous communication[1] we have reported a study of 20 patients suffering from rheumatic fever treated with neocinchophen—the ethyl ester of *para*-methyl phenylcinchoninic acid. This drug was found to exert an antiarthritic and antipyretic action in rheumatic fever comparable to, though slightly less effective than, that of the salicylates. It also produced symptoms of drug intoxication similar to those of salicylism: tinnitus aurium, vertigo, nausea, vomiting, abdominal distress, and signs of renal irritation. These symptoms of toxicity were in general less marked than in salicylate medication. However, it was suggested that as much caution should be exercised in the administration of large doses of neocinchophen as with the administration of sodium salicylate or of acetyl-salicylic acid.

During this clinical investigation of neocinchophen a parallel and more or less comparative study was made of the action of another phenylcinchoninic acid derivative—the ethyl ester of phenylcinchoninic acid.†

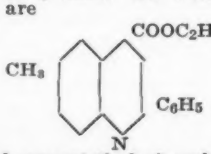
These two drugs, neocinchophen and the ethyl ester of phenylcinchoninic acid, seem to be the most efficacious of a large group of compounds which have been prepared in an effort to secure a derivative as potent therapeutically as phenylcinchoninic acid (atophan, cinchophen), but without its

*From the Hospital of the Rockefeller Institute for Medical Research, New York.
Received for publication, March 22, 1924.

†This is marketed by the firm of Schering and Glatz, Inc., under the trade-name of Novatophan Improved. Its formula and that of neocinchophen are



Ethyl ester of phenylcinchoninic acid:
Novatophan Improved.



Ethyl ester of *para*-methyl phenylcinchoninic acid:
Neocinchophen
Novatophan
Tolysin

irritating properties. The acid itself exhibits three striking pharmacologic actions:

I. It increases uric acid elimination. This property gave it its first clinical value, and caused it to be quickly adopted as a specific in gout.

II. It relieves the symptoms of rheumatic fever in a manner similar to the salicylates.

III. It restrains the inflammatory reaction to local chemical irritation—its so-called antiphlogistic action—as shown by Starkenstein[2] and by Dohrn[3] who found that rabbits receiving phenylcinchoninic acid by mouth, subcutaneously, or intravenously failed to develop the characteristic conjunctivitis when mustard oil was applied to the eyes. Hanzlik and Tainter,[4] on the other hand, claim that this antiphlogistic action is not demonstrable when other methods of producing local irritation are used.

Many of the derivatives of phenylcinchoninic acid differ therapeutically from the acid itself out of all proportion to their differences in chemical composition. Klemperer[5] has shown that their antigout, antirheumatic, and antiphlogistic properties do not run hand in hand. In fact, some of the compounds retain none of these qualities. According to Dohrn the ethyl ester has no antiphlogistic property in the sense of preventing the mustard oil conjunctivitis in rabbits. Very little information is available concerning its action in rheumatic fever.

Method.—Eight patients ill with rheumatic fever were treated with the ethyl ester of phenylcinchoninic acid. A summary of the clinical data is given in Table I. The method of administration was the same as that described for neocinchophen, i.e., 1 gm. of the drug each hour until the appearance of some evidence of therapeutic effect or of drug intoxication, at which time the drug was discontinued for that day. On succeeding days the patient was kept well under the influence of the drug and as free as possible from toxic symptoms. In other words, we endeavored to maintain the maximum subtoxic dosage. This amount varied considerably and had to be determined for each patient, but in general corresponded to the dosage which we found necessary for neocinchophen. The period of administration varied from four to forty-one days.

Six of the 8 patients treated had severe polyarthritis and one mild polyarthritis at the time of administration. In these 7 patients the arthritis disappeared in 24 to 72 hours after the beginning of medication. In four no relapse occurred. In the three other patients the symptoms were only temporarily alleviated and reappeared when the drug was discontinued. Coincident with the relief of the arthritis there occurred a disappearance of fever and rapid heart rate. As with neocinchophen, there frequently was noted a bradycardia during the first week of administration.

One patient (Case No. V), though relieved of his arthritis by previous treatment with neocinchophen and with aspirin, showed definite signs of persistent infection: low-grade fever, rapid pulse, leucocytosis, and horizontal weight curve. Under the ethyl ester of phenylcinchoninic acid he gradually improved.

TABLE I

RESULTS OF TREATMENT

CASE HOSP. NO.	SEX AGE WEIGHT	PREVIOUS ATTACKS. AGE 1ST. ATTACK.	DAY OF DISEASE TREATMENT BEGAN	DRUG†	AMOUNT OF DRUG		THERAPEUTIC EFFECT	TOXIC EFFECT	
					1ST 24 HRS.	TOTAL		CLINICAL SYMPTOMS	URINE
GROUP I: NO RELAPSE AFTER DISCONTINUING DRUG									
I 4718	Male 14 yrs. 51 kgs.	None	7th	Nov. imp.	12 gms. (0.24)*	150 gms. in 30 days	Arthritis, fever, and tachycardia disappeared within 2 days. Bradycardia for 1st 4 days	Slight nausea	Negative before. Ft. tr. albumin and occasional cast throughout administration
II 4658	Male 14 yrs. 49 kgs.	One 5 yrs.	11th	"	11 gms. (0.22)*	140 gms. in 31 days	Arthritis disappeared within 24 hrs. Sharp fall of temp. from 105° to normal on 1st day followed by 6 wks. of low grade fever. Pulse subnormal 1st wk., then gradually developing tachycardia. Evidence of severe cardiac involvement	None	Slight increase in albumin and appearance of occasional cast and many renal epith. cells during administration. Negative after discontinuance
III 4733	Male 18 yrs. 44 kgs.	Nine 9 yrs.	6th	"	9 gms. (0.20)*	19 gms. in 4 days	Arthritis, fever, and tachycardia disappeared within 24 hrs.	None	No increase in albumin or casts
IV 4724	Male 32 yrs. 48 kgs.	One 22 yrs.	119th	"	8 gms. (0.16)*	112 gms. in 26 days	Mild arthritis and fever disappeared within 24 hrs. Pulse slightly rapid for wks.	Nausea and vomiting end of 1st day	Tr. albumin before; no increase. No casts before. Occasional hyaline cast during administration. Many renal epith. cells and few r.b.c. 1st 2 days
V 4513	Male 21 yrs. 58 kgs.	None	29th	Neo.	13 gms. (0.11)*	114 gms. in 14 days	Severe arthritis disappeared within 2 days; mild symptoms for 8 more days. Temp. and pulse normal within 3 days. Severe relapse 2 days after drug stopped; treated with aspirin	Slight nausea 2nd day	Tr. albumin before; no increase. Many renal epith. cells, occasional r.b.c. and hyaline casts 1st few days
			45th	Asp.	10 gms. (0.16)*	177 gms. in 44 days	Fever and tachycardia disappeared within 24 hrs.; arthritis within 3 days. Mild relapse after aspirin was discontinued; treated with the ethyl ester of phenylcinchoninic acid	Anorexia	Tr. albumin, few casts and r.b.c. 1st 2 days of administration. Disappeared on 3rd day

*No. of grams per kilo body weight.
†Nov. imp. = Ethyl ester of phenylcinchoninic acid.
Neo. = Neocinchophen.
Asp. = Acetyl. salicylic acid (aspirin).

Neo. = Neocinchophen.
Asp. = Acetyl. salicylic acid (aspirin).

TABLE I—CONT'D

RESULTS OF TREATMENT

CASE HOSP. NO.	SEX AGE WEIGHT	PREVIOUS ATTACKS. AGE 1ST. ATTACK.	DAY OF DISEASE TREATMENT BEGAN	DRUG	AMOUNT OF DRUG		THERAPEUTIC EFFECT	TOXIC EFFECT	
					1ST 24 HRS.	TOTAL		CLINICAL SYMPTOMS	URINE
			98th	Nov. imp.	12 gms. (0.20)*	137 gms. in 21 days	Low grade fever and leucocytosis persisted; tachycardia gradually diminished. No joint symptoms	None	No albumin. Occasional hyaline casts 1st day only
GROUP II: RELAPSE AFTER DISCONTINUING DRUG									
VI 4628	Male 13 yrs. 39 kgs.	Six 6 yrs.	27th	Nov. imp.	13 gms. (0.33)*	125 gms. in 25 days	Fever disappeared within 12 hrs. Arthritis and tachycardia within 2 days. Severe relapse immediately after discontinuance of drug	Slight nausea and abdominal distress end of 1st day	Ft. tr. albumin before. No increase. No casts
			61st	Asp.	6 gms. (0.15)*	40 gms. in 17 days	Severe arthritis disappeared within 24 hrs. Mild arthritis persisted for 6 days. Fever and tachycardia disappeared within 4 hrs.	Tinnitus, nausea, vomiting, deafness, and vertigo 1st day	Slight increase in albumin, many renal epith. cells and occasional r.b.c. 1st 48 hrs.
VII 4622	Female 44 yrs. 63 kgs.		218th	Nov. imp.	17 gms. (0.27)*	168 gms. in 16 days	Fever disappeared within 12 hrs. Tachycardia and severe arthritis within 48 hrs. Mild stiffness persisted throughout administration. Relapse began 9 days after discontinuance of drug	None	Ft. tr. albumin before; no increase. Many renal epith. cells during administration. Occasional cast during and following administration
			246th	Asp.	8 gms. (0.12)*	52 gms. in 24 days	Fever and tachycardia disappeared within 8 hrs. Arthritis entirely disappeared within 48 hrs.	Slight tinnitus, deafness, vertigo and nausea 1st 4 days	Ft. tr. albumin before; no increase. Occasional casts before; increased 1st 5 days. Many renal epith. cells and occasional r.b.c. 1st 2 days
VIII 4367	Male 19 yrs. 61 kgs.	Three 14 yrs.	6th	Nov. imp.	10 gms. (0.16)*	269 gms. in 41 days	Fever and tachycardia disappeared within 2 days. Arthritis within 3 days, mild arthritis reappeared on 10th day and persisted. Severe relapse occurred while dosage was being decreased	Slight tinnitus and vertigo 1st 2 days	Tr. albumin before; not increased; disappeared during administration. Occasional casts 2nd day only
			48th	Asp.	7 gms. (0.11)*	190 gms. in 40 days	Arthritis disappeared within 24 hrs.; fever and tachycardia within 48 hrs.	Tinnitus, deafness, vertigo. nausea and vomiting 1st 2 days	Negative before. Tr. albumin 1st 2 days only. Occasional casts throughout administration

This drug also gave rise to toxic symptoms similar in character to those of salicylism but milder in degree. While no definite general conclusions can be drawn from this small series of cases the possibility is suggested that the ethyl ester of phenylcinchoninic acid is less toxic than neocinchophen.

One patient, Case No. V (mentioned above), and the three who suffered relapses after medication was withdrawn and were subsequently treated with aspirin, furnished the opportunity to compare as to therapeutic efficiency and toxicity the ethyl ester of phenylcinchoninic acid and aspirin—one of the established salicylate antirheumatics. The comparison corresponds closely to that of neocinchophen and the salicylates.[1] In brief, the latter seem to be somewhat more effective in relieving the symptoms of rheumatic fever and at the same time more toxic.

CONCLUSION

The ethyl ester of phenylcinchoninic acid corresponds closely to neocinchophen as an antisymptomatic remedy for rheumatic fever.

REFERENCES

[1]Boots, Ralph H., and Miller, C. Philip: Jour. Am. Med. Assn., 1924, lxxxii, 1928.
[2]Starkenstein, E., and Wiechowski: Prag. med. Wchnschr., 1913, xxxviii, 36.
[3]Dohrn, Max: Die Therapie der Gegenwart, 1913, liv, 196.
[4]Hanzlik, P. J., and Tainter, M. L.: Jour. Lab. and Clin. Med., 1923, ix, 166.
[5]Klemperer, G.: Die Therapie der Gegenwart, 1913, liv, 257.

ORGANIC, PROTEIN AND COLLOID SILVER COMPOUNDS

III. Does the "Colloidal Silver" Become Available as Antiseptic?*

By Torald Sollmann and J. D. Pilcher, Cleveland, Ohio

INTRODUCTION

As has been shown in the previous papers of this series,[1] the growth of yeast cells is restrained by silver ions. The immediate inhibiting action of the various "colloidal" and "protein" silver compounds is due to the silver ions which are present in some degree in all the compounds of this class. When these are deducted, there remains a very considerable amount of silver which, under the conditions of the experiment, takes no part in the antiseptic effect and which may be designated as "inactive silver" (Table I). It consists mainly of mixtures of metallic silver and of silver oxides, perhaps also of obscure silver protein compounds, all in colloidal form.

*From the Department of Pharmacology, of the Medical School of Western Reserve University, Cleveland.

This investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

Received for publication, March 24, 1924.

TABLE I

PERCENTAGES OF ACTIVE AND INACTIVE SILVER IN SOME OF THE MORE IMPORTANT COMPOUNDS

COMPOUND	TOTAL SILVER CONTENT	ACTIVE SILVER	INACTIVE SILVER
	%	%	%
Silver Nitrate	63.5	63.5	0
Albargin	15.0	13.3	1.7
Protargol	8.3	7.5	0.8
Collargol	78.	1.5	76.5
Argyrol	22.5	0.4	22.1
Silvol	20.0	0.2	19.8

Assuming it to be established that this colloidal silver plays no part in the immediate antiseptic action, there remains the possibility that it might become gradually converted into active ionic silver, either by spontaneous progressive decomposition of the colloids, or to replace such ionic silver as is bound or precipitated in the course of the antiseptic action. This theory, that the colloidal silver constitutes a "reserve" of potential silver ions, which prolongs the antiseptic efficiency, prevails quite widely, i.e., while it is universally conceded that the colloidal silver compounds are much weaker than silver nitrate, when the contact is short, it is often asserted that they become (relatively) much more active if the contact is prolonged. It must be pointed out, however, that this assumption is essentially speculative; no objective evidence that would stand reasonable criticism is available.

The present investigation was intended to answer this question; i.e., whether the colloidal silver constitutes "reserve-silver," that might become available under suitable conditions; or whether it remains inert, serving merely as a colloidal demulcent.

The problem was approached from several angles, as will be seen.

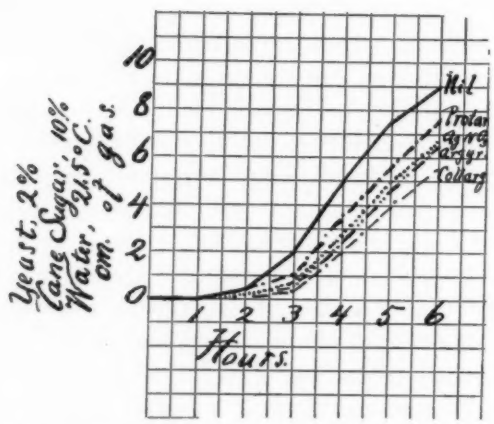
I. THE INFLUENCE OF TIME OF CONTACT ON THE RELATIVE EFFICIENCY OF SILVER COMPOUNDS

If the colloidal silver is capable of gradual transformation into antiseptically active silver, its importance would increase with the duration of the contact of the yeast with the silver compound; i.e., the efficiency ratio: $\frac{\text{colloidal compound}}{\text{silver nitrate}}$ would increase with the duration of the experiment.

To test this matter, the method previously described was modified so that six hours, instead of one hour, would be required to form 10 cm. of gas. This is easily accomplished by reducing the percentage of yeast; for it was found that the time required to produce a given quantity of gas varies approximately as the reciprocal of the concentration of yeast, the sugar percentage remaining constant; i.e., if it is wished to slow the gas formation to one-half or one-fourth, it suffices to reduce the percentage of yeast to one-half or one-fourth. The agreement is sufficiently close. Another method for slowing the gas formation consists in reducing the temperature. In our principal series, the two measures were combined; incubating 2 per cent yeast with 10 per cent cane sugar at 21° C. (instead of the 4 per cent of

yeast at 38° C., used in the standard one-hour method). This yields 10 cm. of gas in six hours. Hourly observations were made. Four different concentrations of compounds with widely different proportions of colloidal silver (see Table I) were used; namely, silver nitrate, protargol, argyrol, and collargol.

The point of the experiments, then, was this: If the colloidal silver becomes gradually activated, the ratio of gas formed in the colloidal compounds, as compared with that formed in silver nitrate, should *decrease* from the earlier hours to the later hours. If, on the other hand, the two run parallel, or if the ratio increases with the time, then the colloidal silver has remained inert.



Curve 1.—Rate of gas formation with different silver compounds.

Note that the curves are essentially parallel; if the reserve silver had become active, the curve would be relatively high in the early periods, and relatively low in the later periods.

———— Unpoisoned solution.
. . . . . Silver-nitrate, 0.1 mg.
- . - . - . - . Protargol 1.5 mg.
- . . - . . - Argyrol 10 mg.
- - . - - . - - . Collargol 5 mg. (all per 10 c.c. of mixture).

The outcome of all the series of experiments concurs in showing that the ratio of gas formation does *not* decrease with time; i.e., they show that the *colloidal silver remains inert;* and this whether the concentration of active ions is relatively low or relatively high; whether the experiments are made

TABLE II

QUANTITY OF GAS LIBERATED IN WATERY MIXTURE OF YEAST 2 PER CENT, CANE SUGAR 10 PER CENT, INCUBATED AT 21.5°C:

	CM. OF GAS			RATIO OF GAS, COMPARED WITH $AgNO_3$=1		
HOURS	2	4	6	2	4	6
Products and dosage (mg. per 10 c.c.):						
$AgNO_3$ 0.1 mg.	*	2.7	6.5	1.0	1.0	1.0
Protargol 1.5 mg.	0.3	3.5	7.5	*	1.3	1.2
Argyrol 10. mg.	*	2.5	6.5	1.0	0.9	1.0
Collargol 5 mg.	0	2.0	5.5	–	0.74	0.85
Control unpoisoned	0.3	5.0	10.0	*	1.8	1.5

*Present, but too small for measurement.

TABLE III.

AS IN TABLE II, BUT NORMAL SALINE IN PLACE OF WATER.

HOURS	CM. OF GAS			RATIO OF GAS, COMPARED WITH $AgNO_3$=1		
	2	4	6	2	4	6
Product and dosage (mg. per 10 c.c.):						
$AgNO_3$ 2 mg.	1.5	4.5	8.0	1.0	1.0	1.0
Protargol 10 mg.	0.4	5.5	10.0	0.27	1.2	1.3
Argyrol 5 mg.	0.8	4.5	8.5	0.53	1.0	1.1
Collargol 10 mg.	0.3	2.7	6.0	0.2	0.6	0.67

in water or in normal saline; with 2 per cent of yeast at 21° C.; or with ½ per cent at 38° C.: under none of these conditions does the silver become available.

For illustration, we may quote the figures of curves of a typical experiment in water (Table II and Curve I) and in normal saline (Table III).

The figures are quoted only for 2 hour intervals for the sake of simplification.

It must therefore be concluded that the nonionic silver of colloidal silver compounds does not become converted into ionic silver during six hours of contact with yeast mixtures; it is therefore antiseptically inert, and cannot be considered as "reserve-silver."

II. THE QUANTITY OF SILVER AVAILABLE AFTER COMPLETION OF THE STANDARD EXPERIMENT

It may be assumed that the active, i.e., ionic silver produces its effects by combining with the protoplasm of the yeast, and that it is thereby rendered inactive. This is confirmed by the observation that a given quantity of silver can paralyze only a limited quantity of yeast (E. Zerner and R. Hamburger).[2]

The "active" silver can, therefore, be promptly and more or less completely removed by digesting with yeast. "Reserve-silver" would not be affected by this, except in proportion as it became converted into active silver.

If, therefore, a just-inhibitory dose of a silver-compound is added to a yeast mixture and digested for an hour, the active silver will be more or less completely bound, but the greater part of any "reserve-silver" would be still available. If, then, fresh yeast is added and again digested, the gas formation in the second digestion would be restrained distinctly more by preparations containing utilizable "reserve-silver," than by those in which no silver reserve is mobilized; and if a preparation contains utilizable reserve-silver, its antiseptic efficiency in the second fermentation should be greater than with silver nitrate.

The experiments were conducted in the usual manner, by digesting at 38° C., a 4 per cent suspension of yeast in 10 per cent cane-sugar solution, containing the just-inhibitory concentrations of the silver compounds, and observing the gas formation at the end of an hour. The tubes were then emptied, and the mixture shaken, or rubbed with 1 or 4 per cent of fresh

yeast. They were then re-mounted, digested for a second hour, and again observed.

When 4 per cent of yeast was added in the second digestion, the gas formation was so active that practically all the tubes were completely filled with gas; so that no quantitative conclusions could be drawn.

The average figures for the other experiments are shown in Table IV; the individual data agreeing well with the averages.

TABLE IV

GAS FORMATION IN YEAST MIXTURES ON 1ST. AND 2ND. DIGESTION

	FIRST DIGESTION	SECOND DIGESTION	
	4% YEAST	WITHOUT FURTHER YEAST	WITH 1% OF FRESH YEAST
	(A)	(B)	(C)
Silver nitrate 0.45 mg. per 10 c.c.	0.6 cm.	2.0 cm.	4.5 cm.
Protargol, 4.0 mg. per 10 c.c.	0	1.6.	3.5
Argyrol 35 mg. per 10 c.c.	0	0.5	3.75

Column C shows somewhat less restraint by silver nitrate than by the other compounds, in the second digestion; but the silver nitrate was also somewhat less efficient in the first digestion and this accounts fully for the small difference in the second digestion.

This line of experimentation therefore leads to the same result as the first line; i.e., that the "colloidal silver" in the silver compounds is useless as an antiseptic.

CONCLUSIONS

The "colloidal silver" of the "colloidal and protein-silver compounds" does not exert any antiseptic action even after six hours of contact; the entire antiseptic efficiency resides in the silver ions that are present or become immediately available. Whatever value the colloidal silver has must depend upon demulcent qualities, which would serve to diminish the irritative effects of the antiseptic silver ions. It is therefore very doubtful whether the colloidal part of the silver compounds plays any greater part in their therapeutic action than would other indifferent colloids such as the proteins that occur in the same compounds.

BIBLIOGRAPHY

[1]Pilcher, J. D., and Sollmann, T.: JOUR. LAB. AND CLIN. MED., 1923, viii, 301; erratum, ibid., p. 462; 1924, ix, 256.

[2]Zerner, E., and Hamburger, R.: Biochem. Ztschr., 1921, cxxii, 315.

A QUANTITATIVE COMPARISON OF THE TOXICITY OF THE ALKAMINE ESTERS OF AROMATIC ACIDS USED AS LOCAL ANESTHETICS*

By Charles W. Hooper, A.B., M.D., and Elsa Becker, A.B., New York City

ACCURATELY controlled animal experiments showing the relative toxicity of the various drugs used as local anesthetics may have a direct clinical application in supplying unbiased data by which the relative safety of the drugs may be estimated.

The toxicity of the local anesthetics has been so widely discussed recently that we will confine ourselves to the results we have obtained by a method which from an experimental viewpoint may be considered quantitative.

Professors Hatcher and Eggleston, 1919, were the first to carry out comprehensive experimental work to determine the relative toxicity of all of the drugs used as local anesthetics. They realized that the toxic action of the local anesthetics is exerted only after the entrance of the drugs into the blood stream and therefore that the most accurate method of determining the relative toxicity of the local anesthetics is to determine the maximal toxicity of each after intravenous administration. They determined the maximal toxicity of each by rapid intravenous injections of water solutions of the drugs into cats. The concentration of the solutions varied from one to twenty per cent so that the dose could be injected from a syringe in from five to fifteen seconds.

In view of the fact that the ratio of toxicity of each of the local anesthetics varies greatly in different species of animals when injected subcutaneously, we decided to determine the maximal toxicity by intravenous injection in some species other than the cat and also to see whether we could develop a method which from an experimental viewpoint might be considered quantitative. For this purpose we adopted a test based upon the official method used by the Hygienic Laboratory, United States Public Health Service, and the manufacturers to determine the toxicity of arsphenamine and related products before they are released for distribution. The Government selected the rat for this test because the results obtained seemed to be more constant than with other experimental animals. Furthermore, on account of their small size, a large number of rats can be kept conveniently under uniform conditions of diet, weight, etc., and as a result more animals can be used for an individual test.

We have applied a modification of the official technic for the determination of the maximal toxicity of the alkamine esters of the aromatic acids

*From the Department of Experimental Medicine, H. A. Metz Laboratories.
Received for publication, March 24, 1924.

used as local anesthetics and have found that the results obtained are very accurate and correspond very closely to those obtained with the cat by Professors Hatcher and Eggleston.

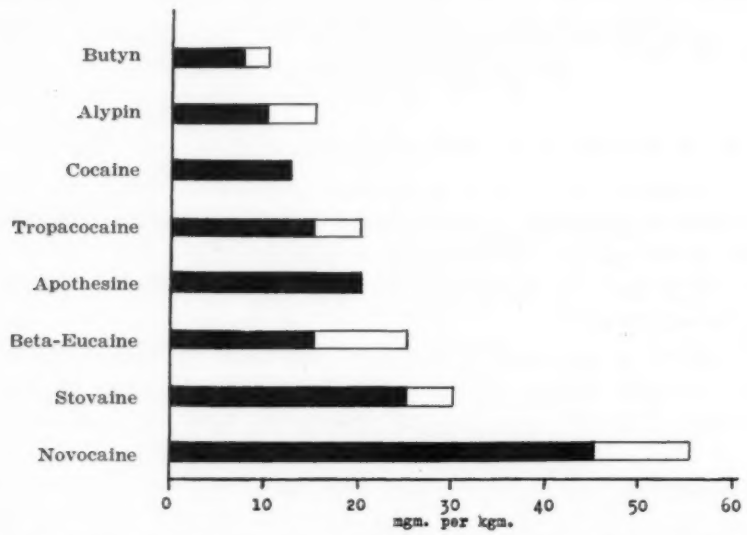


Chart 1.—A Quantitative Comparison of the Toxicity of the Alkamine Esters of Aromatic Acids used as Local Anesthetics. Maximal Tolerated and Minimal Lethal Dose in Milligrams per Kilo Body Weight.

TECHNIC OF THE TEST

Healthy nonpregnant albino rats weighing between 100 and 150 grams were employed. The rats were obtained from one source and were kept on a constant diet for at least two weeks before the tests. They were fasted for from sixteen to twenty hours immediately before the injection of the drugs when they were weighed and the doses administered per kilo body weight. Water was supplied during the fast period. A 2 per cent solution

TABLE I

COMPARATIVE VARIATION IN THE TOXICITY RESULTS OBTAINED IN THE ALBINO RAT AND CAT AFTER INTRAVENOUS INJECTION OF THE ALKAMINE ESTERS OF THE AROMATIC ACIDS USED AS LOCAL ANESTHETICS

	ALBINO RATS		CATS	
	MAXIMAL TOLERATED AND MINIMAL LETHAL DOSES	STRENGTH OF SOLUTION	FATAL DOSE	STRENGTH OF SOLUTION
	mg. per kg.	per ct.	mg. per kg.	per ct.
BUTYN	7.5-10	2	15*	1
ALYPIN	10 -15	2	10**	5-10
COCAINE	12.5	2	15**	5-10
TROPACOCAINE	15 -20	2	18-22**	10
APOTHESINE	20	2	20**	5-10
BETA-EUCAINE	15 -25	2	10-12.5**	5
STOVAINE	25 -30	2	25-30**	10
NOVOCAINE	45 -55	2	40-45**	5-20

*Fatal dose obtained by Prof. Robert A. Hatcher. See Bonar, M. L., and Sollmann, Torald: Jour. Pharmacol. and Exper. Therap., 1922, xviii, 475.

**Fatal doses obtained by Prof. Cary Eggleston and Prof. Robert A. Hatcher: Jour. Pharmacol. and Exper. Therap., 1919, xiii, 444.

TABLE II

A QUANTITATIVE COMPARISON OF THE TOXICITY OF THE ALKAMINE ESTERS OF THE AROMATIC ACIDS USED AS LOCAL ANESTHETICS. RATS INJECTED INTRAVENOUSLY. PERCENTAGE OF RATS THAT DIED. (THE NUMBERS WITHIN PARENTHESES REPRESENT THE NUMBER OF RATS USED IN THE SERIES)

	DOSE EXPRESSED IN MILLIGRAMS PER KILO BODY WEIGHT													
	5	7.5	10	12.5	15	17.5	20	25	30	40	45	50	55	60
	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
BUTYN	0(6)	20(5)	70(10)		100(5)		100(5)							
ALYPIN	0(5)		14(7)		71(7)		100(7)							
COCAINE			0(5)	60(5)	80(5)		80(10)	100(5)	100(5)					
TROPACOCAINE			0(5)		20(5)		100(5)							
APOTHESINE					0(5)	0(5)	80(5)	100(5)						
BETA-EUCAINE			0(5)		20(5)		40(5)	100(5)						
STOVAINE							0(5)	20(5)	100(9)					
NOVOCAINE									0(5)	0(14)	17(30)	25(40)	57(23)	75(24)

of the drug in distilled water was injected into the saphenous vein at the rate of twelve to fifteen seconds for every 0.1 c.c. At least five rats were injected at each dose. The animals were kept under observation for twenty-four hours, during which time they were fed the routine diet. The minimal dose at which any of a series of animals died is considered the maximal tolerated dose. The dose at which the majority of a series died is considered the minimal lethal dose.

The abscissae represent the number of milligrams of the drugs injected per kilo body weight. The shaded portions of the ordinates show the maximal tolerated doses, the unshaded portions the minimal lethal doses. The chart shows that butyn is the most toxic of the alkamine esters of the aromatic acids used as local anesthetics. The maximal tolerated dose is 7.5 milligrams and the minimal lethal dose 10 milligrams. The maximal tolerated dose of cocaine as well as the minimal lethal dose is 12.5 milligrams. The maximal tolerated dose of apothesine as well as the minimal lethal dose is 20 milligrams. The maximal tolerated dose of novocaine is 45 milligrams and the minimal lethal dose is 55 milligrams.

In the first column are given the local anesthetics employed; in the second column the maximal tolerated and minimal lethal doses for the albino rat in milligrams per kilo body weight; in the third column the strength of the solution injected into the saphenous vein of the rat; in the fourth column the fatal dose for the cat obtained by Professors Hatcher and Eggleston by their rapid intravenous injection method and in the fifth column the strength of the solution injected.

It will be noted that the results obtained by the two methods are quite comparable and that there is very little variation in the maximal toxicity for the two species when the drugs are injected intravenously.

It will be noted that the toxicity results obtained by injecting the local anesthetics intravenously into albino rats by the above described method are clean cut. The difference in the individual susceptibility of the animals is slight. The variation between the maximal tolerated dose and the minimal lethal dose does not exceed ten milligrams in any of the experiments. The results indicate that the method from an experimental viewpoint may be considered quantitative.

LABORATORY METHODS

AN ADJUSTABLE DOUBLE-SLIT LAMP FOR USE IN MULTIPLE OPTICAL REGISTRATIONS*

By Louis M. Katz, A.M., M.D., and William R. Baker, Cleveland, Ohio

INTRODUCTION

THE successful employment of optical manometers and similar types of apparatus involves not only a considerable amount of experience but also requires careful attention to a large number of details before satisfactory photographic records can be obtained. Efforts are therefore being made in this laboratory to simplify the practical aspects of such registration. The adjustable double-slit lamp described in this communication was constructed in order to facilitate the projection of light beams upon mirrors of several optical recorders, in such a way as to avoid parallax in the reflected beams. This lamp has been successfully employed during the past year.

PRINCIPLES OF OPTICAL REGISTRATION

To understand the advantages of the lamp it is necessary to recall the essential principles which underlie optical registration. The clearness of optical records is determined to a large extent by the arrangement of the three essential parts of an optical system, namely, the recording camera, the projecting lamps and the reflecting mirrors on the manometers or segment capsules. A good curve is obtained when the beam of light striking the camera slot is focused and has sufficient luminosity, and when the movements of the reflecting mirror are adequately magnified. The focus of the beam is readily adjusted by shifting the position of the projecting lens. The luminosity of the reflected beam depends (a) on the type of lamp used, (b) on the distance between the mirrors and the camera, (c) on the proximity of the mirrors to the focus of the source of light, (d) on the width of the reflecting mirrors.

It has been the experience in this laboratory that the best magnification is obtained, with manometers and segment capsules of proper vibration frequency, when the distance between the mirror and camera is approximately 120 cm. In order to maintain a proper vibration frequency, relatively small mirrors (2 to 4 mm. in width) must frequently be used. At this distance, and with such small mirrors, the light value of a Nernst lamp is insufficient except for very slowly moving photographic paper. The only source of light

*From the Physiological Laboratory of the Medical School, Western Reserve University, Cleveland.
We are indebted to Professor Todd and his assistants for the photographs shown in this article and to Mr. Joseph Dvorak for the line drawings.

so far available under these conditions has therefore been the D.C. arc lamp, a very useful model of which has been devised by Wiggers.[1]

It is often necessary, both in the clinic and the research laboratory, to record more than one curve at the same time. In general, such optical records are obtained either by using several lamps, one for each manometer or segment capsule, or by using a single lamp constructed to project more than one beam of light. In comparing the time relationship of such simultaneously recorded curves the question of parallax must be taken into account. And probably nowhere is this so important as in the modern exact optical studies of cardiodynamics. The duration of the phases of the heart cycle are so brief and occur in such rapid succession that even a slight uncorrected parallax may lead to a significant error. A little reflection will convince one that the presence or absence of parallax depends entirely on

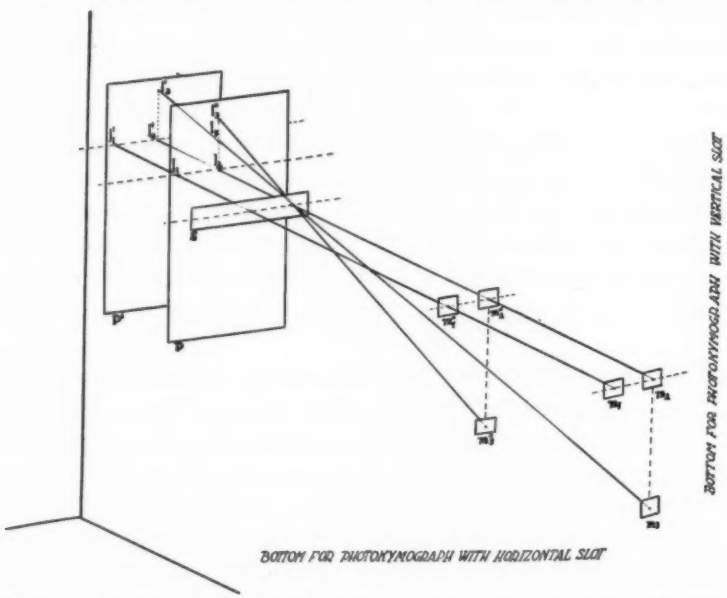


Fig. 1.—A three plane diagram showing how the relation of the mirrors and photographic surfaces to the camera slot modifies the amount of parallax. S represents the camera slot; P and P', two positions of the photographic surface; I_1, I_2 and I_3, the images of beams of light reflected respectively from mirrors m_1, m_2 and m_3 on the photographic surface P; I'_1, I'_2 and I'_3, the images from the same mirrors on photographic surfaces P'_3; I''_3, the image of beam from mirror m''_3 on photographic surface P. The images from m''_1 and m''_2 are the same as those from m_1 and m_2. Further description in text.

the arrangement of the reflecting mirrors with regard to the axis of the cylindrical lens of the photokymograph. Parallax is absent when the centers of the reflecting mirrors are in the same plane as the axis of the camera slot (m_1 and m_2, m''_1 and m''_2 in Fig. 1); this is to say the mirrors must be horizontal when the camera slot is horizontal and over one another when the slot is vertical. Parallax is present *only when the above condition is not fulfilled* and its amount depends on (*1*) the displacement of the mirrors from the plane of the camera slot, (*2*) the distance between the mirrors and the lens of the photokymograph and (*3*) the distance between the lens and the photographic surface. The greater the displacement of the mirrors from the plane of the camera lens, that is the greater the distance between m_2 and

m_3 in Fig. 1, the larger will the parallax become because the angle between the two beams will increase. The amount of parallax will also be increased if the mirrors are brought closer to the camera (compare images of m_2 and m_3 with those of m''_2 and m''_3 in Fig. 1) or if the photographic paper is moved away from the lens of the camera (compare I_2 and I_3 with I'_2 and I'_3 in Fig. 1). In the former case this is due to a greater angle between the two beams of light and in the latter to the greater separation of the beams as they travel back.

The significance of inattention to parallax is not always the same but depends on the rapidity with which the photographic surface moves past the slot, for the time value of each linear unit is much less at rapid rates than at the slower ones. The above statements can be summed up in the following formula:

$$p = \frac{f.m}{r.s}$$

in which p is the time value of the parallax; f, the distance from lens to photographic paper; m, the distance between the two mirrors in a plane parallel to the photographic surface and perpendicular to the axis of the camera lens; r, the average distance from the recording mirror to the camera lens; and s, the speed with which the photographic surface moves past the camera slot.

For practical reasons it has not always been possible to obviate parallax and therefore various methods have been introduced for evaluating it. For the most part they consist either in photographing the beams of light when the photographic surface is stationary, preferably through amber glass (Garten[2]) or in simultaneously interrupting the light beams while the records are being obtained.

CRITIQUE OF OTHER LAMPS

How do the various types of optical systems used in the past compare according to these principles? Otto Frank[3] recognized that the recording of simultaneous curves can be simplified by using a single lamp which projects more than one light beam. He accomplished this by providing a Nernst filament lamp with a triple objective. The beams of light from his lamp are focused on the several mirrors either by approximating or separating the mirrors (a procedure not always possible) or by varying the distance between the mirrors and the lamp. In other words, the amount of adjustment with this lamp is rather limited. C. Tigerstedt[4] modified the "Frank" lamp in two essential details for this reason. His lamp has five objectives instead of three, each of which is movable independently of the others. A wider range of adjustment is therefore possible with Tigerstedt's lamp but yet not sufficient for all purposes. Moreover, parallax cannot be avoided when using either of the lamps, a fact that these investigators recognized and corrected by interrupting the several light beams simultaneously.

H. Straub[5] obtained a double beam of light from a single lamp by another principle. He projected two closely placed parallel Nernst filaments through a single objective and claimed that in this way he avoided parallax entirely,

or at least reduced it to a negligible factor. Inasmuch as the two filaments are fixed, Straub's lamp has to be adjusted in the same way as Frank's and therefore has the same disadvantages. In addition to the drawbacks just mentioned all of the Nernst filament lamps lack the luminosity of the arc lamp and moreover they do not permit a variation in the width of the beams.

The arc lamp constructed by Wiggers,[1] which projects a band of light by means of a slit of adjustable width, can also be focused on several mirrors. It allows a good deal of adjustment of the mirrors particularly in the axis of the light band. Parallax is always present, however, because the axis of the light band is at right angles to the axis of the camera slot and therefore does not allow one to place the mirrors in the plane of the camera slot. Wiggers has minimized the amount of parallax to 0.75 mm. when the

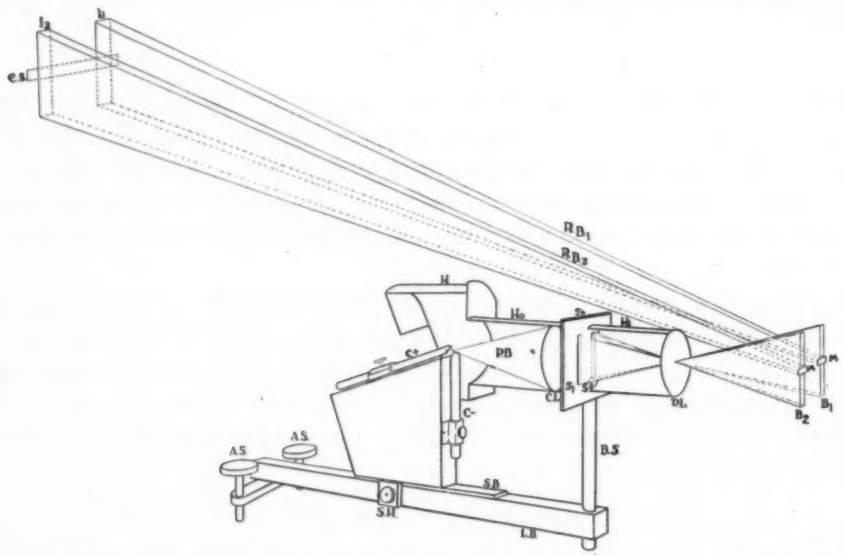


Fig. 2.—A three plane diagram of the double-slit lamp showing the important details of its construction. The side of the hood H and the draw tubes H_0 and H_1 toward the observer have been removed in this diagram in order to show the course of the light beams. Description is given in text.

mirrors are 120 cm. from the camera by placing a narrow hood 16 cm. long in front of the camera slot. The parallax in the system is determined by Garten's method.

THE ADJUSTABLE DOUBLE-SLIT LAMP

The adjustable double-slit lamp which we built and which is described in this communication combines the valuable features of the preceding lamps and at the same time obviates many of their difficulties. With it we are able to project two parallel bands of light, to adjust the width of each of the bands *independently* and above all *to vary the distance between them.* The luminosity of this lamp is sufficient even at a distance of 120 cm. and with bromide paper moving past the camera slot at a rate of 135 mm. per second as proved by actual test; an efficiency reached only by Wiggers' arc lamp. There is no difficulty with focusing and there is a large margin for adequate magnification of the curves. Parallax can readily be avoided simply by see-

ing that the two mirrors lie in the plane of the camera lens. In fact we have calculated by the formula given and proved by actual experiment that the amount of parallax produced when the mirrors are separated 1 cm. in a plane at right angles to the camera slot, is less than 0.15 mm. The ability to shift the bands of light independently allows a greater freedom in the placement of the manometers than can be attained with any other lamp heretofore described, a feature of great significance when studying the pressure changes in the various heart chambers. Adjustments of the various manometers (or capsules) can be made without throwing everything out of line; all that is required is to shift the band of light concerned to a new position.

A schematic drawing of the lamp and the course of the light beams is shown in Fig. 2. The principle employed is to bring the light which passes through the two variable slits S_1 and S_2 on the exact optical center of a projecting lens (P.L.) by means of a condensing lens (C.L.) and then to allow the light to spread as two bands B_1 and B_2 to be reflected from two mirrors (M) as rays RB and RB_2. The details of the lamp are as follows: A 4-ampere 110 D.C. Spencer arc lamp (C−, C+) is mounted on a bar (S.B.) which slides on a long steel bar (L.B.) 2.5 cm. in diameter and 53 cm. long. The lamp is fixed in position by tightening a set screw (S.H.). The whole apparatus may be tilted at various angles by the set screws (A.S.). These are 9 cm. in length and are set in a cross bar which is fastened to the back of the long steel bar. Each of the screws is held in position by means of a lock nut to enhance rigidity.

The projection system which consists essentially of a condensing lens (C.L.), a projecting lens (P.L.) and an adjustable double-slit mechanism (Sh.), is supported in a circular flat steel clasp resting on the top of the upright bar (B.S.). With this arrangement the projection system can be revolved around its long axis so that the bands of light can be projected perpendicular to the camera slot at all times, regardless of whether the slot is vertical or horizontal.

The condensing lens is an ordinary biconvex lens 5 cm. in diameter with a principle focus of 9 cm. It is used to bring the light (P.B.) from the positive carbon (C +) to a focus in the center of the projecting lens (P.L.). In other words the arrangement is such that the light from the two slits comes to a point in the optical center of the projecting lens, regardless of their width or position. Spherical and chromatic aberration are prevented in this way even when inexpensive lenses are employed.

The projecting lens is also biconvex and has the same diameter and focus as the condensing lens. It is mounted in a telescoping draw tube (H_1) in front of the slit mechanism and it can be placed at any distance from 10 to 18 cm. in front of the slits by adjusting the draw tube. This adjustment allows the images I_1 and I_2 to be focused sharply on the camera slot (C.S.).

The distance between the slit mechanism and the condensing lens is fixed at 4 cm., the light from the positive carbon being focused on the projecting lens by shifting the position of the arc lamp on the long bar. The space between the arc lamp and the condensing lens is made light-tight by a tele-

scoping draw tube (H_0) which allows the lamp to be placed anywhere from 9 to 35.5 cm. behind the lens.

The principal axis of the entire optical system is parallel to the long bar (L.B.) and 15 cm. above it. In most of our work we have set the system at the following distances: Positive carbon to condensing lens 21.5 cm.; condensing lens to slits 4 cm.; slits to projecting lens 10 cm.; projecting lens to mirrors 10 cm.; mirrors to camera lens 120 cm.; camera lens to photographic paper 1 cm.

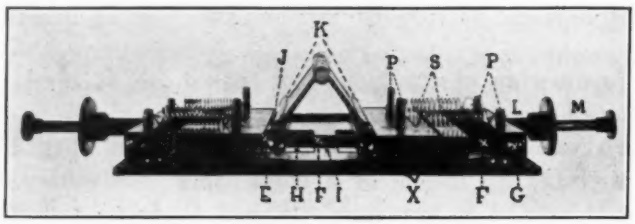


Fig. 3.—Side view of the shutter mechanism described in text.

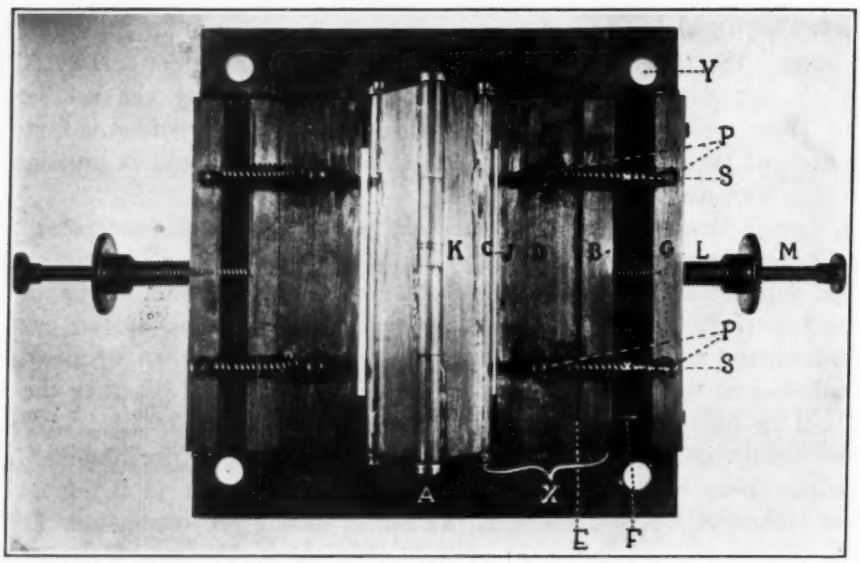


Fig. 4.—Front view of the shutter mechanism described in text.

THE DOUBLE-SLIT SHUTTER MECHANISM

The construction of an adjustable double-slit mechanism constituted a mechanical problem of the greatest difficulty. Figs. 3 and 4 are introduced to make clear the details of its structure as it was finally built. It consists of a brass aperture plate (A) 7.5 cm. square and 1.6 mm. thick, on which are mounted six sliding bars, 3.5 mm. thick and 5.5 cm. long; the two outer bars (B) being 4.8 mm. wide, the two inner ones (C) 2.8 mm and the intermediate bars (D) 12.7 mm. The aperture plate has two oblong openings (H), 12 mm. by 40 mm., which are separated by a rib of metal (I) 4.8 mm. wide. The inner and outer bars on each side are connected at the top and bottom

by two tubes (E) 3.2 mm. in outer diameter and 1.6 mm. bore, the distance between the bars being fixed at 19.2 mm. This constitutes the sliding frame (X). The two tubes of this frame slide on two parallel round rods (F) which are 1.6 mm. in diameter and 7.5 cm. long. The rods are mounted so as to form an angle of exactly 90° with the rib of the aperture plate and are fastened at either end to a bar (G) which is screwed to the aperture plate. The intermediate bar (D) slides on the tubing of the sliding frame. The space between the inner and outer bar forms the slit opening on each side (J), the under surface of the bars adjoining the slit being beveled to form a knife edge. The space between the inner bars of the two sliding frames is bridged by a metal hinge (K) which forms a light-tight partition and prevents light from passing through this space.

The position and width of each slit can be altered *independently* by means of four thumb screws (L and M), two on each side, which work against four tension springs (S). The tension springs are fastened to pins (P) placed in the slide rod supports (G) and the intermediary bar (D). This arrangement allows the springs to exert an outward pull both on the intermediary bar and the sliding frame (X). The outer thumb screw (L) which is hollow is threaded through the slide rod support (G) and presses against the outer side of the outer bar (B). When it is screwed in, it forces the sliding frame and with it the slit towards the mid-line. When it is screwed out the sliding frame is pulled away from the mid-line by the tension springs. The inner thumb screw (M) passes through the hollow outer thumb screw (L), is threaded into the outer bar (B) of the sliding frame and presses against the intermediate bar (D). When it is screwed in, it forces the intermediate bar towards the mid-line narrowing the slit. When it is screwed out the tension springs pull the intermediate bar outward and widen the slit.

The width of each slit (J) can be varied from 0 to 1 mm. The distance between the slits can be varied from 6.4 to 25.4 mm. When the mirrors are placed 10 cm. in front of the projecting lens it means that the mirrors can also be shifted a like amount.

The aperture plate of the shutter mechanism is fastened to a plate on the end of the metal tube which holds the condensing lens. A piece of asbestos 3.5 mm. thick is interposed between these plates in order to prevent the conduction of heat to the shutter mechanism by the metal. The telescope tube holding the projecting lens has a plate at its free end which is also fastened to the aperture plate by four threaded pins at (Y). A space of 2 cm. is, however, left behind the shutter mechanism which allows a free circulation of air and helps to prevent an excessive rise in its temperature. For the sake of clarity these attachments have been omitted in the diagram given in Fig. 2.

REFERENCES

[1]Wiggers: Circulation in Health and Disease, 1923, ed 2, p. 179.
[2]Garten: Tigerstedt: Handbuch der Physiol. Methodik, 1911, i, 103.
[3]Frank: Zeitschr. Biol., 1913, xlvi, 519.
[4]Tigerstedt, C.: Skan. Arch. f. Physiol., 1913, xxviii, 37.
[5]Straub: Deutsch. Arch. f. klin. Med., 1914, cxiii, 535.

A NEW UNIVERSAL OPTICAL MANOMETER*

By Carl J. Wiggers, M.D., and William R. Baker, Cleveland, Ohio

IT is a relatively simple matter to construct an optically recording manometer which is sufficiently sensitive to record the details of intraventricular and aortic pressure changes and still has the requisite inherent vibration frequency to reproduce them with accuracy. It is a far more difficult task, however, to design the instrument so that it is satisfactory in experimental work. To do this the anatomic difficulties in its application, the proper alignment in relation to illuminating lamps and camera and a certain flexibility in adjusting the reflected beams horizontally as well as vertically have to be taken into consideration, not to mention the necessity of maintaining an adequate "light value" in the reflected beams.

In 1914, one of us[1] described a form of optical manometer which represented so great a technical advantage over existing forms that it has proved the instrument of choice in our laboratory ever since. In spite of several minor changes leading to simplification this instrument still has certain disadvantages which not only necessitate a certain amount of skill in its operation but which also somewhat restrict its uses.

Briefly these disadvantages are as follows:

1. In order to obtain a vertical and horizontal adjustment of the reflected beam, a second tilted and rotating mirror was employed. As a first surface mirror is not feasible (owing to accidental sprinkling with blood and solutions) the intensity of the reflected beam is so greatly reduced that good records cannot be obtained at paper speeds greater than eighty mm. per second. On the other hand, on slow paper and on film, good results are often frustrated by the reflection of double images (i.e., both from the front and silvered surfaces of the mirror).

2. The manometer can only be used with photokymographs having a vertical slot and horizontally moving paper, a disadvantage because the cameras available commercially have all been developed in connection with string galvanometers and therefore have horizontal slots and a vertical movement of the paper or film.

3. Inasmuch as the manometer cannot be tilted far from a vertical position after insertion and as the segment surface cannot be altered independently of the entire manometer, it is technically difficult and at times impossible to place manometers close enough together to record pressures from adjacent points in the cardiovascular system. This also prevents their illumination by a single source of light such as described by Katz and Baker thus entailing the necessity of making careful and repeated checks as to possible errors of parallax.

*From the Physiological Laboratory of the Medical School, Western Reserve University, Cleveland, Ohio.

The new model obviates all of these disadvantages, is easier to operate successfully and not only yields better contrast in records at similar speeds but can be used with bromide paper traveling up to 150 mm. per second.

As shown in Fig. 1, the apparatus consists of a vertical glass tube 1.6 cm. in diameter and 8 cm. long, closed above by a stopcock and bulb. To the stem of the latter a calibrating manometer may be attached and through this avenue a small quantity of anticoagulation fluid may also be introduced in order to prevent or dislodge accidental clots in the cannula. At the bottom, the standard cannula fittings described in the older form are attached. The segment capsule fixture is attached to a short lateral T-tube 4 cm. from the bottom of the glass manometer tube.

To this is attached the metal fixture providing for a movable segment, capsule surface (*A*), 4 mm. in diameter. This fixture is constructed as fol-

Fig. 1.

lows: The segment capsule with a groove for fastening the rubber dam is fitted to a hollow brass ball (*B*). This is accurately ground to make a ball and socket joint with the metal attachment cemented to the short lateral glass tube and is firmly held in place by a lock ring (*C*). By means of this swivel joint, the entire capsule and its mirror can be adjusted in any direction by a convenient handle (*D*). Not only does this permit slight shifting of the light beams reflected to the camera slot, thus dispensing entirely with a second mirror arrangement, but by turning the ball through an angle of 90° the chord side can be swung to the right and the apparatus used with vertical beams and with cameras in which the paper moves vertically. The advantage of this arrangement in avoiding errors of parallax has been emphasized in a preceding paper by Katz and Baker.

In order to project a base line a second small mirror is carried on a small platelet (*E*). This is connected with the hollow ball (*B*) and segment

capsule in such a way that the mirrors attached to the rubber of the segment capsule and the platelet respectively, move together as the ball is rotated by the handle (*D*). Independent adjustment of the "base line mirror" is also possible, however, by another but smaller swivel joint controlled by a handle (*F*). When final adjustments have been completed the large ball is securely locked by tightening the ring (*C*) thus making disarrangement during the course of an experiment impossible.

The manometer has been tested practically during the past year in a diverse series of experiments, both with simpler forms of arc-lamps and with the double-slit mechanism described by Katz and Baker. These tests have demonstrated that this instrument contributes greatly to simplify the technic of optical registrations of dynamic phenomena of the heart and circulation.

REFERENCE

[1]Wiggers: Am. Jour. Physiol., 1914, xxxiii, 382.

THE IMPROVED NEUBAUER RULING FOR BLOOD CELL COUNTING*

By Simeon Trenner, Philadelphia, Pa.

THE Neubauer ruling has now generally supplanted the various rulings hitherto used in hemacytometry, as it combines all the advantages of the original Thoma, with its cell depth of 0.1 mm. and its central counting area of one square millimeter subdivided into 400 small squares for counting red cells, with an additional area of eight square millimeters, the four corner squares of which are subdivided into sixteen small squares to facilitate the counting of white cells.

For many years it was the custom to count the red cells in either the Thoma or Neubauer rulings in groups of twenty-five $\frac{1}{400}$ mm. squares, but it is now the almost universal practice to count these cells in groups of sixteen squares. This method saves time and insures greater accuracy as no computation is required, it being only necessary, with a dilution of 1:200, to add four ciphers to the count of eighty small squares.

To meet the wishes of the advocates of both methods, and possibly because of manufacturing difficulties at that time, the small squares were divided in the Thoma and in the original Neubauer rulings into groups of both sixteen and twenty-five by the addition of an extra line in the middle of every fifth square (see Figs. 1 and 1-*A*).

The need for a simple boundary occupying less space than this and less confusing in use was suggested in a personal communication by Roger S. Morris, M.D., University of Cincinnati. This improvement, based upon the suggestion of Morris and now embodied in the improved Neubauer ruling, consists in the division of the 400 small squares comprising the central square millimeter into twenty-five groups of sixteen by a new "split" line.

*From the Laboratory of Cytometry, Arthur H. Thomas Company, West Washington Square, Philadelphia, Pa.

This appears under the microscope as a transparent boundary line for each group of sixteen small squares (see Figs. 2 and 2-*A*) and, since the confusing line through the middle of each fifth square has been eliminated, the area of the entire 400 squares is available for counting instead of 256 as in the original Neubauer.

The counting of cells, with a 16 mm. objective, in groups of sixteen small squares instead of in larger groups of twenty-five squares permits the use of the center of the field.

Some of the advantages of this improvement in ruling, in combination with the technic recommended for its use, are as follows:

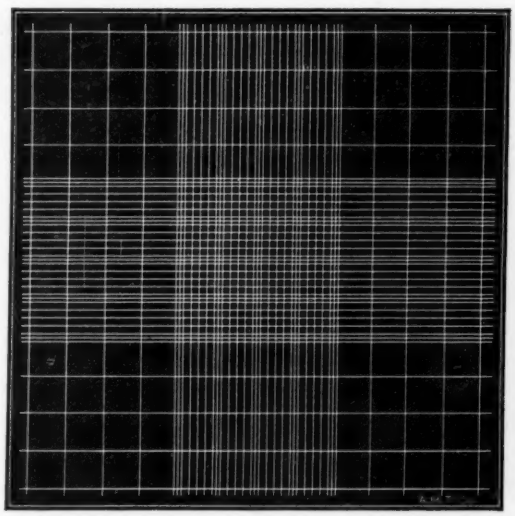


Fig. 1.—Entire area of *original* Neubauer ruling in which the groups of 16 smallest squares are separated by an extra line in the middle of every fifth square.

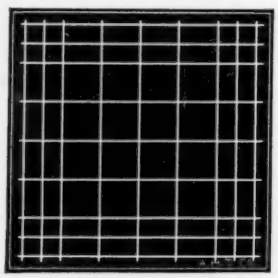


Fig. 1-*A*.—Group of 16 smallest squares of *original* Neubauer ruling showing boundary by fifth squares with extra line in middle of each.

There is no confusion in orienting and grouping the lines and cells in counting, as the worker passes from group to group with ease and certainty.

There is less eye strain in continuous counting as the field is small, flat, and evenly illuminated and the observer is not compelled to keep his eye close to the ocular or rigidly in the optical axis.

Continuous manipulation of the fine adjustment is unnecessary as the ruling is in sharp contrast to the extreme corners of the group and all the cells and squares are in perfect focus simultaneously.

Higher eyepiecing is possible without removal of spectacles than when a larger group of twenty-five squares is counted. "Sweating" of the eye lens and the resultant loss of definition is to a great extent prevented.

The draw tube can be extended for increased magnification when necessary without undue distortion or breaking down of the image.

Photographic records can be made with a 16 mm. achromatic, 0.65 N. A., objective that are sharp to the corners of the group.

The technic of counting is quickly conveyed by the demonstrator and grasped by the student.

The entire central square millimeter is conveniently visible in the field of the 16 mm. objective, just as in the Thoma and original Neubauer rulings.

The four corner square millimeters are, as in the original Neubauer, each subdivided into sixteen squares for convenience in counting white cells.

The improvement in ruling involves no basic change in the usual counting technic, as the same subdivisions of the nine square millimeters of counting area of the original Neubauer ruling, are retained.

With the improved Neubauer ruling the count is made from the center of the white or transparent line—visible as a result of the splitting of the line bordering each group of sixteen small squares—to the next engraved line when counting the cells in one square, or to the center of the next transparent line when counting the cells in a group of sixteen squares, employing the usual rule regarding cells touching the lines on two adjacent sides.

If the worker prefers metric computation by counting in groups of twenty-five squares, this can be accomplished by counting the cells in the nine small squares bordering on two adjacent sides of the split boundary

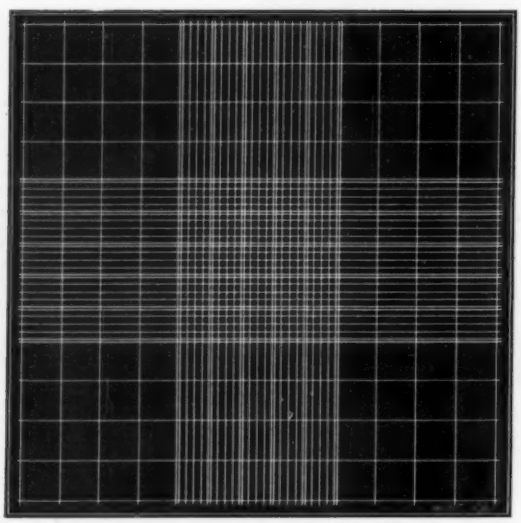


Fig. 2.—Entire area of *improved* Neubauer ruling, showing split boundary lines.

Fig. 2-*A*.—Group of 16 smallest squares of *improved* Neubauer ruling showing split boundary lines.

line of the group of sixteen squares. The counting of four such groups widely separated gives the same metric red cell counting unit as in the Thoma and original Neubauer rulings. The same result is also attained by counting the cells in five rows of twenty small squares each horizontally across the field.

Cells can be counted by any of the several methods in general use; i.e., horizontally across the field, diagonally, or the center and corner squares, with increased facility because of the absence of the confusing extra line in every fifth square.

When the greatest possible accuracy is desired, a double chamber is used, with a drop from each of two pipettes, and the cells counted in the entire area of both rulings. This can be done with greater convenience than with the original Neubauer and Thoma rulings as there is no bisection of the peripheral rectangles in the improved Neubauer ruling.

QUANTITATIVE METHODS FOR THE DETERMINATION OF FORMIC ACID IN BLOOD AND URINE*

By F. De Eds, San Francisco, Cal.

METHODS for the determination of small quantities of formic acid in blood and urine are essential in studies of the fate of formaldehyde, hexamethylenamine, and methyl alcohol in the organism. Such methods should permit the determination of very small quantities of formic acid in order to ascertain with a reasonable degree of certainty the possible occurrence of formaldehyde in low antiseptic concentrations from the hydrolysis of hexamethylenamine, and the possible conversion of methyl alcohol to formic acid. Methods answering these requirements are not available in the literature, and the methods that have been used are not only not sensitive enough, but also inaccurate, thus rendering our present knowledge of the fate of formaldehyde and its compounds, and of methyl alcohol insecure, and a reinvestigation of the entire subject desirable.

The object of this paper is to demonstrate the unreliability of the methods of Pohl,[1] and Autenrieth[2] for the determination of formic acid in urine, and to propose methods which are applicable to blood and urine for the determination of such small quantities of formic acid as 0.12 mg. in 25 c.c. of blood, and 1.2 mg. in 150 c.c. of urine, corresponding to about 1:200,000 and 1:125,000, respectively. Before proceeding to a description of the proposed methods, the older methods will be discussed.

OLDER METHODS

The principal quantitative methods for formic acid described in the literature depend upon the reduction of salts of silver, or of mercury, the product of reduction then being estimated by gravimetric or volumetric processes. The reduction of mercuric chloride to calomel is typical of these methods.

1. *Method of Autenrieth for Urine.*—Three hundred c.c. of urine are treated with 30 c.c. of 25 per cent phosphoric acid, and placed in a 750 c.c. Jena glass flask with a few pieces of broken porcelain. The flask is connected with a condenser for direct distillation. Heating is started with a small flame to prevent frothing, the intensity of the heating being gradually increased. Three hundred c.c. of distillate are collected in a graduated cylinder. Distillation is stopped, 300 c.c. of water added to the residue and distillation resumed. This process is repeated until the distillate is no longer acid, this point being reached when a total of 1,400 to 1,500 c.c. of the distillate are obtained. The combined distillates are now treated with an excess of calcium carbonate, and evaporated on

*Submitted in partial fulfillment of the thesis (Part I) requirements for the degree of Doctor of Philosophy.

From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco.

Received for publication, May 14, 1924.

the water-bath to about 20 c.c. After evaporation, the solution is filtered and the filter paper washed thoroughly. Fifty to sixty c.c. of a saturated aqueous mercuric chloride solution are added to the filtrate, and the mixture heated on the water-bath for five to six hours, reduction of the mercuric chloride to calomel taking place. One to three c.c. of 20 per cent hydrochloric acid are now added, and the heating on the water-bath is continued ten minutes longer. After cooling, the precipitated calomel is collected on a weighed filter paper, washed with distilled water until free from chlorine, then with alcohol and ether, and finally dried at 100 to 110° C. to a constant weight. One gram of calomel is equivalent to 0.0977 grams of formic acid.

Using this method, Autenrieth[2] reported the presence of 0.5 to 0.65 gm. of formic acid in 48 hour samples of urine, the urine volume ranging from 3,000 to 4,800 c.c. Six such determinations were reported by him on normal individuals, in two of the instances control determinations in good agreement being made. Autenrieth claimed that his results showed formic acid to be a constituent of normal urine.

2. *Method of Pohl for Urine.*—The method of Pohl,[1] which was published before that of Autenrieth, is essentially the same as that of Autenrieth, the only difference being that steam distillation is substituted for direct distillation.

In justification of the applicability of the method, Pohl presented the following data: an aqueous solution containing 0.3750 gm. of sodium formate was analyzed, and 0.3754 gm. was found. One hundred c.c. of urine were analyzed and found to contain 0.0141 gm. of sodium formate. Sodium formate, 0.5779 gm., was added to another 100 c.c. portion of the same urine, and 0.5801 gm. was found after making allowance for the blank value. A repetition of this experiment by Pohl, using 70 c.c. quantities of urine, again gave good recovery of added formate.

Pohl does not state whether the value obtained on blank urine was due to formic acid, or to some other reducing substance. Assuming that the blank value was due to formic acid, Pohl's value on 70 c.c. of urine should agree fairly well with the blank value obtained on a 100 c.c. sample, and moreover these values should approximate the values obtained by Autenrieth. Converting the blank value found on 70 c.c. for comparison on the basis of 100 c.c. the following is found: 0.0763 gm. $Hg_2Cl_2 \times 10/7 = 0.109$. Therefore, the difference in weight of calomel yielded by two 100 c.c. quantities of urine is 0.011 gm., or multiplying by 0.0977, 0.001 gm. of formic acid. Assuming the urine volume for 48 hours to be 3 000 c.c., the formic acid excreted, as indicated by Pohl's data, would be 0.2871 gm. This value differs greatly from the value of 0.5 gm. or more reported by Autenrieth. To be sure, Autenrieth reports lower formic acid values in various pathologic conditions, but so little is known about this entire question that the possibility remains that his values on urine may be due to the presence of reducing substances other than formic acid. Autenrieth states that aldehydes and sulphurous acid reduce mercuric chloride to calomel, but are eliminated during the analysis. However, it will be pointed out later that there is another reducing substance in urine, and also in blood, not removable by the methods of Autenrieth and Pohl.

From all this it is indicated that the methods of Autenrieth and Pohl are uncertain for giving quantitative recovery of formic acid from urine. Nevertheless, fifteen trials with both methods, as described above, were made on pure solutions and on several different urines containing formic acid. The urines were always fresh and obtained from healthy subjects in the laboratory. Typical data illustrative of the results that were obtained are presented in Table I.

These data show that the controls, or blank urines, gave variable values, rendering the methods unreliable. This is especially true of the last three results, which were obtained on urines collected successively from the same individual. The large blank value on a urine collected immediately before taking hexamethylenamine makes the small value obtained after taking the drug meaningless. This variability found by me is in agreement

TABLE I

DETERMINATION OF FORMIC ACID IN PURE SOLUTIONS AND IN URINES BY METHODS OF AUTENRIETH AND POHL

URINE OR SOLUTION USED	METHOD USED	FORMIC ACID ADDED	FORMIC ACID FOUND; CALCULATED FROM CALOMEL
		gm.	gm.
25 c.c. of formic acid solution	Autenrieth	0.1311	0.1368
25 c.c. of formic acid solution	Pohl	0.1311	0.1152
A. 300 c.c. urine containing formic acid	Pohl	0.1311	0.2288
B. 300 c.c. of same urine (blank)	"	None	−0.1435
			0.0853
A. 300 c.c. urine containing formic acid	Autenrieth	0.1311	0.3269
B. 300 c.c. of same urine (blank)	"	None	−0.1749
			0.1520
*300 c.c. urine containing formic acid	Pohl	0.1311	0.6112
*300 c.c. of same urine (blank)	"	None	−0.4754
			0.1358
*300 c.c. of urine after taking 3 gm. of hexamethylenamine by mouth	Pohl		
	"	None	0.1346

*These three urines were from the same individual, the last sample being an aliquot portion of a urine collected after taking 3 gm. of hexamethylenamine by mouth, the collection of urine being continued until excretion of the drug was complete, as indicated by a negative test with the phloroglucine reagent for formaldehyde applied to a sample of urine after treatment with acid.

with that found in the data reported by Autenrieth and by Pohl. Further evidence that urine possesses an extremely variable blank value, which may be interpreted as formic acid, is in the report of Sollmann,[3] who found that the quantity of sodium formate in control or blank urines of the first period of the day (i.e., between 6:30 and 10 A.M.) varied from 0.28 to 3.37 mg. per 100 c.c. In several instances the residues obtained by me were so large and so decidedly different from calomel that the estimations were stopped, the results being obviously erroneous, despite every precaution used in carrying out the methods.

It is concluded from all that has been said, and the results that were obtained, that the methods of Autenrieth and Pohl for the estimation of formic acid in urine are unreliable.

3. *Method of Stepp and Zumbusch for Blood.*—Owing to the lack of success with the methods of Autenrieth and Pohl, determination of formic acid in blood was resorted to, using a slight modification of the method proposed by Stepp and Zumbusch[4] for blood. The modification consisted of the use of steam distillation instead of the vacuum distillation recommended by these authors. In all other details the procedure was that of Stepp and Zumbusch, and as follows:

A blood filtrate was prepared by precipitation of the protein with phosphotungstic acid, the excess phosphotungstic acid being removed with neutral lead acetate. Steam distillation of the filtrate was then performed, the distillate being received into a solution of sodium hydroxide. The distillate was evaporated to a small volume and the formic acid estimated by the method of Riesser.[5] This consisted of heating the solution containing the formic acid (10 to 20 mg.) with considerable excess of a solution containing 200 gm. $HgCl_2$, 300 gm. sodium acetate, and 80 gm. of NaCl per liter for 6 hours on a water-bath. The mixture was cooled, and without filtering from the precipitated calomel, was treated with 10 c.c. of 25 per cent HCl. Then an excess of concentrated aqueous potassium iodide solution (4 gm. KI for every 10 c.c. of above mercuric chloride reagent used) was added followed by an excess of N/10 iodine solution. The calomel was thereby converted to mercuric chloride. The clear solution was titrated with N/10 sodium thiosulphate solution,

using starch as indicator in the usual way. The number of cubic centimeters of N/10 iodine required multiplied by 0.0023 represented the amount of formic acid in the volume taken for analysis.

A great many trials were made with this method without obtaining satisfactory results. Successive determinations on the same blood failed to give check results. In most instances, metallic mercury in varying amounts was one of the products of reduction, and in the presence of metallic mercury the thiosulphate titrations are obviously meaningless. However, the titration feature of the method was recognized as desirable and was made a part of the method to be proposed. The chief difficulty with blood was to obtain a filtrate free from reducing substances other than formic acid.

Various methods of obtaining such a blood filtrate were tried. Precipitation of the protein with 95 per cent alcohol, with absolute alcohol, with absolute alcohol followed by the addition of a saturated alcoholic solution of zinc chloride proved inadequate when the modified Stepp and Zumbusch procedure was applied to the filtrates. Check determinations were not obtained, and reduction of mercuric chloride to metallic mercury usually occurred. Finally, it was found that treatment of the blood with picric acid completely eliminated the difficulties encountered with other precipitants, and permitted the estimation of 0.1 mg. of formic acid in 25 c.c. of whole blood. The following procedure was adopted for blood.

PROPOSED METHODS

1. *Blood.*—The sample of blood (10 c.c. to 25 c.c.) drawn into a pipette moistened with saturated oxalate is placed in a glass stoppered cylinder of 100 c.c. capacity and diluted to 50 or 60 c.c. with distilled water. The cylinder is stoppered and inverted several times to insure good mixing and hemolysis. After removing and washing the stopper carefully with a few cubic centimeters of water, 5 gm. of picric acid are added. The cylinder is gently rotated until the picric acid sinks. The sides of the cylinder are then washed down with distilled water and the volume made up to the 100 c.c. mark. The cylinder is now stoppered and shaken vigorously until any emulsion formed is broken up, and there is no further change in color. The final color should be a muddy yellow. The material is thrown upon a large filter, and the filtrate collected in a 100 c.c. cylindrical graduate. Filtration is continued until dripping from the funnel ceases. A clear yellow filtrate results. Fifty c.c. of the filtrate are transferred to a 300 c.c. Florence flask, and 5.0 c.c. of mercuric chloride reagent added. The composition of this reagent is the same as that of Fincke[6] and consists of the following ingredients: mercuric chloride 10 per cent, sodium chloride 10 per cent, and sodium acetate 15 per cent in distilled water. Then the mixture is heated on the water-bath for 2 hours and the amount of calomel formed is determined volumetrically as follows: The flask is cooled in running water, and 10 c.c. of 25 per cent hydrochloric acid are added, followed by 4 gm. of potassium iodide and 10 c.c. of N/10 iodine solution. The flask is rotated until all the calomel disappears, by conversion to the soluble mercuric chlo-

ride. The excess iodine is then titrated with N/10 sodium thiosulphate from a microburette of 5 c.c. capacity, using starch as indicator. The end point is the change from green to yellow, and is quite sharp by daylight, less so by artificial light. The calculation is as follows:

c.c. of N/10 iodine x 2 x 0.0023 = gm. formic acid per volume of blood taken.

This method was applied to horse serum, whole beef blood, and whole dog blood before and after the addition of known amounts of formic acid. Typical data are presented in Table II.

TABLE II

DETERMINATION OF FORMIC ACID IN BLOOD AND SERUM

BLOOD OR SERUM (25 c.c.)	FORMIC ACID ADDED	FORMIC ACID FOUND	PER CENT RECOVERY
	gm.	gm.	
Whole Beef Blood	none	none	none
Whole Beef Blood	0.00470	0.0055	117
Whole Beef Blood	0.00115	0.00137	114
Whole Beef Blood	0.00012	0.00013	108
Horse Serum (preserved)	none	none	none
Horse Serum (preserved)	0.00115	0.00115	100
Horse Serum (preserved)	0.00115	0.00111	96.6
Horse Serum (preserved)	0.00230	0.00230	100
Horse Serum (preserved)	0.00230	0.00229	100
Whole Dog Blood	0 00050	0.00050	100
Whole Dog Blood	0.00012	0 00011	91.7

It is seen that amounts of formic acid as low as 0.00012 gm. in 25 c.c. of whole blood or serum can be satisfactorily determined, or in other words, a concentration of about 1:200,000. The blood and serum blanks gave no values for formic acid, indicating that picric acid effects a complete removal of reducing substances other than formic acid in these fluids. Accordingly, the sensitivity and accuracy is great enough for all purposes necessary in studies on the distribution of formate, and the fate of formaldehyde and its compounds, and of methyl alcohol in the organism. However, before the method could be applied in such studies it was necessary to ascertain to what extent these agents will give positive results with the proposed method and whether or not they can be removed. Interference from formaldehyde and hexamethylenamine was expected, but not from methyl alcohol. The latter expectation was fully confirmed.

APPLICATION OF THE METHOD IN PRESENCE OF METHYL ALCOHOL, FORMALDEHYDE AND HEXAMETHYLENAMINE

Methyl alcohol was boiled with mercuric chloride reagent, and gave no production of calomel. Formaldehyde yielded calomel, and likewise hexamethylenamine, since heating facilitated hydrolysis of hexamethylenamine with the liberation of formaldehyde. However, hexamethylenamine was quantitatively precipitated by picric acid and so removed. Formaldehyde was removed by adding 1 c.c. of 10 per cent ammonia to the blood before the addition of the picric acid. Hexamethylenamine was thus formed and precipitated upon the addition of the picric acid. Confirmatory evidence for

the effectiveness of these steps was obtained by the following experiments: One-tenth gram of hexamethylenamine was added to 25 c.c. of whole beef blood, picric acid added, and the analysis carried out as described above. No formic acid was found. A second 25 c.c. portion of the same blood was treated with 0.2 c.c. of 40 per cent formaldehyde and 1 c.c. of 10 per cent ammonia. No formic acid was found upon analysis. A third portion was treated with 0.2 c.c. of 40 per cent formaldehyde, 1 c.c. of 10 per cent ammonia, and 0.0005 gm. of formic acid added. Analysis showed the presence of 0.00051 gm. of formic acid, or, in other words, complete removal of the formaldehyde.

2. *Urine.*—During the trials with the methods of Autenrieth and Pohl on urines, the observation was repeatedly made that reduction of mercuric chloride frequently went to the stage of metallic mercury. Later it was observed that this reduction to metallic mercury was greater, the greater the creatinine content. Having noted this, urea, uric acid and creatinine potassium picrate were investigated for their reducing action by boiling with the mercuric chloride reagent. Out of all these substances creatinine potassium picrate was the only one which caused reduction of mercuric chloride, and the reduction went to the stage of metallic mercury. Consequently, the removal of creatinine from urine was sought for, by making use of the principle found to be successful for analysis of blood, that is, by precipitation with picric acid, and as follows:

One hundred to 150 c.c. freshly voided and 25 c.c. portions of concentrated urines were treated with 5 gm. of picric acid, and shaken vigorously for different time periods. The details of numerous attempts to standardize the time of shaking and process of filtering will be omitted. In brief, the removal of creatinine as the picrate was not uniformly quantitative. Therefore, steam distillation of the picrate urine mixture was resorted to and the following procedure adopted.

One hundred to 150 c.c. of *fresh* urine are placed in a one liter Erlenmeyer flask, 5 gm. of picric acid added and the flask rotated vigorously for ten minutes. The flask is then connected for steam distillation and a distillate of not less than 2000 c.c. collected, 1 c.c. of 20 per cent sodium carbonate being placed in the receiving flask in order to neutralize the formic acid distilled over. The distillate is then transferred to a large evaporating dish and evaporated to about 100 c.c., preferably on a water-bath. The concentrated distillate is then transferred to a Florence flask of 500 c.c. capacity and made slightly acid to litmus with a few drops of 25 per cent hydrochloric acid. Then 5 c.c. of the mercuric chloride reagent used in the blood method are added. The rest of the procedure is the same as in the method proposed for blood and described above. Titration of the calomel is used since it is less troublesome than the gravimetric method. This method was applied to twelve urines. Typical results are presented in Table III.

Table III shows that 1.2 mg. of formic acid added to 150 c.c. of fresh urine may be satisfactorily determined. Since urine to which no formic acid was added gave no formic acid values, and since the principle of precipita-

TABLE III

DETERMINATION OF FORMIC ACID ADDED TO URINE

URINE	FORMIC ACID ADDED	FORMIC ACID FOUND	PER CENT RECOVERED
	gm.	gm.	
100 c.c. fresh urine alone	none	none	none
100 c.c. fresh urine containing formic acid	0.00405	0.00412	101
100 c.c. fresh urine containing formic acid	0.00405	0.00395	97.5
100 c.c. fresh urine containing formic acid	0.00405	0.00360	88.8
150 c.c. of another urine	none	none	none
150 c.c. same urine containing formic acid	0.0040	0.00400	100
150 c.c. same urine containing formic acid	0.0020	0.00210	105
150 c.c. same urine containing formic acid	0.0081	0.0080	98.7
150 c.c. same urine containing formic acid	0.0012	0.00115	95.8

tion with picric acid was used, it seems logical to conclude that the variable results, obtained with the older methods in the first part of the paper, are due to creatinine carried over in variable amounts during distillation.

USE OF PRESERVATIVES

The use of fresh urine is necessary because old urines were found to give definite values when no formic acid had been added. When working with old urines the reduction of mercuric chloride did not go to metallic mercury, hence the reduction was caused by something other than creatinine. Bacterial decomposition may have yielded reducing substances not removed by the picric acid. McGuigan[7] claims that on standing the formic acid content of urine is increased. However, results obtained on urine preserved with chloroform showed that this increase in formic acid on standing urines may be prevented. This matter was tested as follows: A sample of urine was divided into three equal portions, *A*, *B*, and *C*. *A* was analyzed fresh, no formic acid being added. *B* was preserved with chloroform and analyzed two days later. *C* was analyzed at the same time as *B*, but had not been

TABLE VI

TITRATION VALUES FOR FORMIC ACID IN FRESH, PRESERVED AND STANDING URINES

URINE OR SOLUTION	N/10 SODIUM THIOSULPHATE C.C. REQUIRED	FORMIC ACID VALUES; AND REMARKS
10 c.c. Iodine	10.10	
A. Fresh urine	10.11	none; no calomel
B. Urine preserved 2 days with $CHCl_3$	10.09	none; no calomel
C. Urine standing 2 days without $CHCl_3$	8.94	0.0026 gm.; calomel present

preserved with chloroform. The titration results obtained are presented in Table IV, and are self-explanatory, indicating that no formic acid was found, except in the unpreserved urine. Chloroform, therefore, was a suitable preservative.

The use of thymol, cresol, formaldehyde, etc., as preservatives is contraindicated because of a reducing action on mercuric chloride.

DISCUSSION

Autenrieth regarded the values obtained on normal urine by his method as due to a formic acid content. Pohl obtained values on normal urine but not of the same magnitude as those found by Autenrieth. Sollmann reported a large variation in formic acid content of the urine. Using the picric acid method proposed in this paper, a formic acid value has never been obtained on fresh urine, nor on urine preserved for two days with chloroform. It is, therefore, possible that the values obtained by the older methods are due to creatinine carried over during distillation. The same applies to the original method of Stepp and Zumbusch for blood. These authors report formic acid in normal blood. Here again I have found no production of calomel in normal blood when the picric acid method was used, thus giving further support to the possibility of the values being due to creatinine.

CONCLUSIONS

1. It was found that the small quantities of formic acid in urine and blood cannot be determined satisfactorily by the older methods of Pohl and Autenrieth (urine), and by a modification of the Stepp and Zumbusch method (blood).

2. Methods are proposed with which it is possible to determine satisfactorily such small quantities of formic acid as 0.12 mg. in 25 c.c. of blood, and 1.2 mg. in 150 c.c. of urine.

Thanks are due to Dr. P. J. Hanzlik for criticisms and suggestions during the work.

REFERENCES

[1]Pohl: Arch. f. exper. Path. u. Pharm., 1893, xxxi, 281.
[2]Autenrieth: Münch. med. Wchnschr., 1919, lxvi, 862.
[3]Sollmann: Jour. Am. Med. Assn., 1908, li, 818.
[4]Stepp and Zumbusch: Arch. klin. Med., 1920, cxxxiv, 112.
[5]Riesser: Zeit. physiol. Chem., 1915, xcvi, 355.
[6]Fincke: Zeit. nähr. Genussem, 1911, xxi, 1.
[7]McGuigan: Jour. Am. Med. Assn., 1914, lxii, 984.

A NEW CLINICAL METHOD FOR BLOOD TYPING*

By O. M. Gruhzit, M.S., M.D., and L. T. Clark, B.S., Detroit, Mich.

THE need for blood typing has rapidly extended from large hospital centers to the most distant obscure corners of the earth. Its usefulness has increased manifold from Landsteiner's[1] scientific study of heredity of blood characters to practice of blood transfusion for mechanical, toxemic or idiopathic exsanguination. Further, blood group properties are used for phylogenetic study of races.[2] Ottenberg[3] employs blood group characters in identification of parentage of a child. In surgery, Mason[4] introduced blood typing

*From the Department of Medical Research, Parke, Davis and Co., Detroit, Mich.
Received for publication, April 26, 1924.

for selecting proper donors for skin grafting. Lastly, Gruhzit[5] introduced blood typing for differentiation of eclampsia from nephritic toxemias.

Irrespective of the wide use of blood typing, the methods employed have remained in general the same since the time of Landsteiner's (1901) macroscopic and Brem's[6] (1916) microscopic modification and has been strictly delegated to trained laboratory technicians.

The methods in vogue are time-consuming, expensive, and confined to laboratory workers. The present wide use of blood typing demands a method which could be employed by the general practitioners as well as trained technicians and would save time and expense. With this in view, the authors have devised a capillary tube with a central, spindle bulb, one end of which contains the serum, as shown in Fig. 1, and which embodies the above requirements.

THE METHOD

The bent end of the capillary is filled with a standard serum prepared and standardized in the usual manner. It has been our accepted custom to avoid

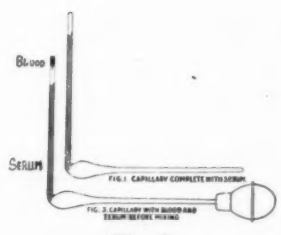


Fig. 1.

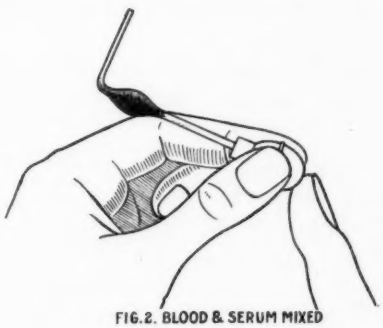


FIG. 2. BLOOD & SERUM MIXED

mixing of sera, to put Serum II in flint glass and Serum III in amber glass capillaries.

Before puncturing the patient's finger or ear, break off the tip of the empty end of capillary, then break off the tip of the end containing serum. This order is essential, as it avoids splashing of serum. Place a small rubber bulb on the empty end of the capillary. Prepare the other capillary in a similar manner. Prick the patient's finger or ear, previously cleansed with alcohol, with a sharp, sterile pin. Squeeze out a minute drop of blood and touch it for an instant with the end of the capillary containing serum. Repeat this with the other tube.

Now grasp the rubber bulb between the left hand thumb and index finger. Place the tip of the right index finger upon the opening in the rubber bulb as shown in Fig. 2. Gradually release the left hand fingers. This draws cells and serum into the spindle enlargement of the capillary. Repeat the compression and release several times. This mixes the cells and serum thoroughly. Repeat this with the second tube.

If the results are read at the place of taking the sample, the capillaries

are set aside for about five to ten minutes, then reagitated and results are noted.

If capillaries are transported a small cork is placed over the tip to avoid spilling.

READING OF RESULTS

Before reading results reagitate contents. If the contents appear granular on agitation and if the flocculation increases on agitation, it means that there is agglutination. Mixture that remains uniform without distinct flocculation to naked eyes, is negative or shows no agglutination.

If it is desired to check up the macroscopic reading with a microscopic, the contents are thrown on plain slide and examined under microscope.

To collect the blood usually takes less than a minute. To read results usually takes five minutes. The test may be completed in five to ten minutes.

There is only one precaution—and that is not to take more of the patient's blood than a mere trace. In fact one never can have too little of blood.

ADVANTAGES OF THE NEW METHOD

The test can be performed by any physician at any place irrespective of laboratory facilities.

The test is simple; results are plainly visible.

The test combines the various steps of the older technic into one.

REFERENCES

[1]Landsteiner: Wien. klin Wchnschr., 1901, xiv, 1132.
[2]Fukamachi, H.: Jour. Immunol., 1923, viii, 291.
[3]Ottenberg, R.: Jour. Am. Med. Assn., 1920, lxxviii, 883.
[4]Mason, J. C.: Jour. Am. Med. Assn., 1918, lxx, 1581.
[5]Gruhzit, O. M.: Am. Jour. Obst. and Gynec., April, 1923, v, 400.
[6]Brem, W. V.: Jour. Am. Med. Assn., 1916, lxvii, 190.

TRANSACTIONS

THIRD ANNUAL CONVENTION AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, ROCHESTER, MINNESOTA

June 5, 1924

The members assembled at the registration desk on the sixth floor of the surgical pavilion, St. Mary's Hospital, Rochester, Minnesota at 8:00 A. M., June 5, 1924. At this desk they were registered and given cards of admission to all of the laboratories and surgical clinics of the Mayo Foundation. As they completed their registration, the members distributed themselves to the laboratories in which they were most interested, particularly the Pathological Department conducted at St. Mary's Hospital. At noon on this day the members were invited by Dr. and Mrs. William J. Mayo to luncheon at their residence. This proved to be a very enjoyable event, and gave members an opportunity to meet each other in a social atmosphere.

At 2.00 P. M. the meeting was called to order in the Assembly Room of the Mayo Clinic and the scientific program began. The following papers were then read:

"Studies in Pollen Sensitization"—J. H. Black, M.D., Dallas, Texas. There was no discussion of this paper.

"A Quantitative Wassermann Test"—E. H. Ruediger, M.D., Los Angeles, California. This paper was discussed by John A. Kolmer, M.D., Philadelphia, Pa.; A. H. Sanford, M.D., Mayo Clinic, Rochester, Minnesota; H. G. Marquez, M.D., San Francisco, Calif.; E. H. Ruediger, M.D. made a few closing remarks relative to his paper.

"Odd Laboratory Appliances"—Francis L. Burnett, M.D., Boston, Mass. No discussion.

"Notes on the Clinical Examination of Protein in Urine with Description of Simple and Rapid Method"—William G. Exton, M.D., New York City. There was no discussion, although several questions were asked of Dr. Exton regarding his paper.

"A Moving Picture Demonstration of the Theory and Practice of the Wassermann Reaction"—C. E. Roderick, M.D., Battle Creek, Mich. No discussion followed.

"Some Physico-Chemical Aspects of Hemolysis"—E. C. Mason, M.D., and P. R. Rockwood, M.D. (By invitation.) This paper was discussed by John A. Kolmer, M.D., and P. R. Rockwood, M.D., also by Drs. Castleman and Brown. The discussions, as well as the papers, were most lucid and interesting. The exchange of views and ideas on the different topics which the papers presented were of unusual value and significance.

There being no further business the meeting adjourned.

President MacCarty called the meeting to order at 8.00 P. M. in the Assembly Room of the Mayo Clinic, a joint meeting with the American Society of Thoracic Surgeons.

Dr. Harry J. Corper of Denver talked on "Personal Experiences with the Cultivation of Tubercle Bacilli and the Use of the Guinea Pig as a Diagnostic Test Animal for Tuberculosis."

Dr. E. C. Kendall, of Rochester, gave the "History of the Discovery of Thyroxin," while Dr. J. G. Hardenbergh, also of Rochester, spoke on the "Care of Experimental Animals." These addresses proved to be most profitable as well as interesting.

The meeting was then adjourned.

June 6, 1924

On Friday, June 6, the meeting was called to order at 8.00 A. M. in the Assembly Room of the Mayo Clinic by President MacCarty.

First, there was a Round Table discussion of the clinical value of laboratory procedures, after which there were discussions on the following topics:

"General Clinical Laboratories" was thoroughly discussed by Dr. A. H. Sanford.

Dr. L. G. Rowntree spoke on "Medical Laboratory Problems."

"Metabolic Laboratories" was the subject of Dr. W. M. Boothby's talk.

"Diabetic Laboratories" was discussed by Dr. R. M. Wilder.

Dr. H. F. Helmholz gave a discussion on "Pediatric Laboratories."

"Surgical Diagnostic Laboratories" was the subject of Dr. A. C. Broders' discussion. Each of these discussions was genuinely appreciated and enthusiastically received by the members.

At 10.00 A. M. visits to the various diagnostic laboratories were made and the members dispersed until afternoon.

At 2.00 P. M. Dr. MacCarty called the meeting to order in the Assembly Room of the Mayo Clinic.

The following papers were read:

"Teaching of Clinical Pathology in the Medical Schools" by Dr. Francis B. Johnson, Charleston, S. C. No discussion.

"A Study of the Specificity of the Kolmer Complement-Fixation Test for Syphilis" by Dr. John A. Kolmer, Philadelphia, Pa. This paper was discussed by Dr. A. H. Sanford, Rochester, Minnesota; Dr. George Ives, St. Louis, Missouri; Dr. E. H. Ruediger, Los Angeles, California; Dr. J. V. Falisi, Fitzsimons Hospital, Denver, Colorado. Dr. Kolmer closed the discussion.

"Several Phases of Spinous Cell Carcinoma" by L. A. Turley, M.A., Ph.D., of Norman, Oklahoma. This paper was discussed by Dr. Philip Hillkowitz of Denver, Colorado. Dr. Turley made the closing remarks.

"Better Postmortem Service" by Dr. H. E. Robertson, M.D., Rochester, Minnesota. (By invitation.) Dr. John A. Kolmer, Philadephia, Pa., and Dr. H. E. Brown, Rochester, N. Y., discussed this paper. Dr. Robertson closed the discussion.

"Interpretation of the More Practical Blood Examinations in Tuberculosis" by Dr. H. G. Sweany, Chicago, Ill. This paper was discussed by Dr. Harry J. Corper of Denver.

"The Practical Value of Examinations of the Saliva in Urea Determination by Dr. P. S. Hench, Rochester, Minn. (By invitation.) Dr. W. G. Exton discussed this paper.

One was aware of the unselfish devotion to their profession of those who, by their long experience, were enabled to submit these various papers for our consideration. The earnestness and zeal with which the papers were discussed was also felt and appreciated.

At 7:00 P. M. the Clinical Pathologists and their wives gathered together for the Banquet at the Kahler Hotel. It is superfluous to add that it was a most charming and entertaining affair.

Not least among the pleasures of the evening were the addresses of Dr. William C. MacCarty, Dr. William J. Mayo and Dr. Leonard Freeman.

Dr. L. B. Wilson delivered an address on "Graduate Education in Clinical Laboratory Branches of Medicine."

Dr. M. T. MacEachern gave an address on "The Clinical Pathologist and the American College of Surgeons."

A feeling of close comradeship was developed and forged at the various gatherings that will prove unforgettable and enduring.

June 7, 1924

Saturday, June 7 at 8:00 A. M. Dr. MacCarty called the meeting to order in the Assembly Room of the Mayo Clinic.

The scientific program commenced. Due to the absence of the authors the following papers were read by title:

"The Influence of the Natural Anti-Sheep Hemolysins of Human Sera Upon the Production of Anomalous Reactions in the First Tube of Kolmer's Quantitative Complement-Fixation Test for Syphilis."—Robert A. Kilduffe, M.D., Los Angeles, Calif.

"Preparation of Jaundiced Patients for Operation." by Waltman Walters, M.D., Rochester, Minn. (By invitation.)

"Pre-cancerous Conditions."—O. J. West, M.D., Seattle, Wash.

"Notes on Treatment of Nasal Diphtheria Carriers."—Clara Israeli, M.D., Manchester, N. H.

Minutes of the previous meeting having been published in the Journal, reading of the same was waived. The President stated that it was not necessary to appoint new committees, and announced that there was no unfinished business.

The Executive Committee, acting as an Auditing Committee, certified the Secretary-Treasurer's books to be correct and their report to that effect was accepted.

The Board of Censors reported orally that they had investigated and approved fifty-one applications for membership and the report was accepted and applicants elected.

The Committee on Standardization of Reagents made no report.

The Committee on Nominations submitted the following:

For president, Dr. John A. Kolmer, Philadelphia, Pa.; for first vice-president, Dr. F. E. Sondern, New York City; second vice-president, Dr. W. F. Thomson, Beaumont, Texas; secretary-treasurer, Dr. Ward T. Burdick, Denver, Colorado.

Dr. Kolmer, upon being nominated for president, made a speech expressing his appreciation of the honor, and in apt terms spoke of the responsibilities the office involved, and of his hesitancy in undertaking the same should he be elected.

All the officers were elected by acclamation.

On motion, a rising vote of thanks was tendered to Dr. William Mayo and Mrs. William Mayo, the Board of Governors of the Mayo Foundation, the Executive Committee, the Ladies Committee and local Committee on Arrangements for the efforts which they put forth for the entertainment of the Society.

On motion the resolution from the American College of Surgeons was accepted.

It was moved to amend one of the by-laws so that it would read as follows: "The Society shall elect annually by ballot at an executive session held during the time of the annual convention," instead of reading, "held on the second day of the annual convention" which motion was carried.

On amended motion, the matter of preparing a leaflet conveying information and instructions on the manner in which specimens are to be sent to the laboratories for examination was referred to the Executive Committee with instructions to submit the same at the next annual meeting of the Society.

It was moved that the committee now in charge of the matter of the standardization of laboratories be continued and that they meet in considering that subject with Dr. L. B. Wilson. Motion carried.

It was moved that the Society present to Dr. L. B. Wilson and his committee, a statement to the effect that The American Sociey of Clinical Pathologists is considering the matter of laboratory standardization, approves of such course or action, and is still considering the best methods by which such object may be attained. Motion carried.

Dr. Hillkowitz, chairman of the Publishing Committee, spoke of the importance of having a Journal that would be interested in the labors and researches of this Society, and that Dr. Warren T. Vaughan, Editor-in-Chief of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, had declared the Journal's intention to emphasize problems that are of practical expediency to clinical pathologists and to include in the editorial staff a greater number of members.

Dr. Burnett, in line with ideas expressed by Dr. Harry J. Corper, suggested that the matter involving the best means of stimulating labor and research work among clinical pathologists, be referred to the Executive Committee for its attention. Motion carried.

It was moved that the place of the next meeting be left to the Executive Committee. Motion amended, designating Philadelphia as the meeting place.

Motion and amendment later withdrawn.

Remarks were made by retiring President MacCarty, who yielded the chair to the new president, Dr. Kolmer, who expressed his appreciation of the confidence reposed in him, and with a few well chosen words of encouragement and inspiration outlined in brief the purposes and main objects of the Society, and spoke in glowing terms of the indebtedness

to Doctors MacCarty, Sanford, Broders and others on the Committee on Arrangements and the Committee on Entertainment, of the Mayo Clinic for the splendid success of the Society's meeting. His views in that behalf met with the most enthusiastic applause.

On motion meeting adjourned until 2.00 P. M.

At 2:00 P. M. the members were conducted in automobiles to the Mayo Farm where they were shown the animal enclosures and various other interesting features.

At 4:00 P. M. there was a pleasant drive in the country which was participated in by the members.

A delightful picnic was held at the Rochester Country Club at 6:00 P. M. and thus ended the third annual convention of The American Society of Clinical Pathologists.

As an authority with a far-sighted vision there are few who will ever exceed Dr. W. J. Mayo, of Rochester. His words of inestimable value to the clinical pathologist, follow here as presented to the attending members the evening of June sixth. It is for those who were not so fortunate as to be able to hear Dr. Mayo, that this is published, as well as for a permanent record for all clinical pathologists.

Dr. W. J. Mayo.—As a general surgeon, I am glad to have this opportunity to express my obligation to the clinical pathologists, who have made possible one of the greatest advances in the surgery of the last decade. In the earlier day, the general surgeon, with the aid of the special senses, especially those of sight and touch, was engaged in gross surgery, usually of the destructive type, owing to the fact that the pathologic processes of which he was able to take cognizance were advanced and, as a rule, indicated radical treatment. Today, through the aid of the various clinical laboratories, the surgeon works with the eye of the microscope, if one may so speak. Pathologic processes are discovered in the early stages of development, with the result that operative procedures can be followed when deviation from the normal physiology is least gross, and surgery has become constructive in its general tendency.

The surgeon can be regarded as a means of mechanical therapy guided by the microscope, and for this guidance in the Clinic we are indebted to MacCarty and Broders and their associates. For example, on opening the abdominal cavity to operate on a neoplasm, a small piece of tissue may be removed for microscopic examination and, within two or three minutes, not only is the nature of the growth known, but also, by the aid of Broders' Index of malignancy, based on MacCarty's work on cell differentiation, the prognosis. If the cells are highly differentiated, the prospect of permanent cure is so great that one is justified in undertaking the serious type of operation, whereas, if the cells are largely undifferentiated, the prospect of cure is so small that a formidable operation would not be justified.

Again, as in carcinoma of the large intestine or rectum, when enlarged glands occur, instead of assuming that the condition is incurable, a gland may be removed for microscopic examination. Often, enlarged glands are the result of sepsis, in which case operation might possibly effect a cure.

Not only is the work of the clinical pathologist important in the operating room, but also in the diagnostic clinic. The diagnosis in a given case may be in doubt. Before starting the patient on a long series of indefinite laboratory examinations which he may be in poor condition to withstand, it is often possible, under local anesthesia, to take a specimen of tissue from an enlarged gland in the groin or supraclavicular fossa, a thickened umbilicus, or from some other indicated situation, to be subjected to the acid test of a microscopic examination which tells truly the nature of the trouble and indicates the proper treatment for it.

The future holds much for you gentlemen, in direct proportion to the recognition of your work by the public and its employment for the benefit of the patient by the medical man.

President MacCarty.—Those of you who know anything about what has happened in this Clinic in the last seventeen or eighteen years, know full well that no one man can know everything about any one subject. It makes no difference how small the subject

may be. In the very early experiences here, when we were trying to learn something about thyroids, occasionally we thought we knew something about them but we learned very soon that our knowledge was limited. We found out early that the only way we could investigate the subject in the Clinic was to have the cooperation of the chemists, physiologists, anatomists, and pathologists; this evening we want to hear from our chemist. This Clinic is very proud of him and his department, which plays a very important rôle in our institution. He hardly needs any introduction because the medical profession has been interested in his work for many years and knows of his splendid achievement in the separation of thyroxin. I thought it would be of interest to you to know something about the evolution of his experiences in connection with thyroxin; I want to present Dr. Kendall, who has charge of this interesting investigation.

Dr. Kendall (Rochester, Minnesota).—Most of you doubtless know of the isolation and identification of thyroxin, and I have not time to give you a recital of the work that has been going on for the past fourteen years. But there are certain details which have come out of the work which show what chemistry can do in a study of the ductless glands, and I should like to have you think of the relation of chemistry to a thing as involved and as far-reaching as the ductless glands. There are clinical classifications of the ductless glands, pathologic classifications and many other classifications, but no one approach to the subject can tell the whole story. There must be some underlying facts by which they are related, and I think that chemistry is going to tell more about that relation than any other science can tell us.

It has been said that universities are made up of persons and traditions, because if you take the traditions and persons away from a university, you have nothing left but the buildings, which in themselves are not of interest; and so it is with chemistry. Chemistry is made up of substances and ideas, but the ideas really are of more importance than the substances. After fourteen years of work in the study of the thyroid, we have a series of chemical substances, some of which are very suggestive and important, but besides that, we have a whole group of ideas, which start with thyroid and reach out through all the ductless glands.

The substance thyroxin was isolated in 1914 in pure crystalline form, and after two years we succeeded in preparing sufficient of it from the thyroid gland to start analyzing it, and finding out what it is chemically. In 1917, this was begun, and for two years we studied the chemistry of the molecule. In 1919 we stopped work with thyroxin itself, and began to try to make it synthetically from carbolic acid, ammonia, and things that are found in the chemical laboratory.

When they found out what adrenalin was, and wanted to make it synthetically, they went to the chemical supply house, and asked for three substances. Those three substances were put together in one molecule, which proved to be adrenalin. When we started out to make thyroxin synethetically, we could not buy three or thirty substances to make that molecule. Thyroxin belongs to a group of compounds which had not been made. For this reason we were forced to spend two years in studying these new compounds, picking up clues here and there, and finally evolving a series of steps which gave us a molecule which we thought should be thyroxin. Thyroxin contains 65 per cent of iodine which is attached to a substance which had never been made synthetically in the laboratory. The family of substances to which this belongs is a new contribution in organic chemistry. The method of the synthesis was to make the compound to which the iodine was attached, and then add the iodine to the molecule and see whether or not the resulting compound was thyroxin. We cheered ourselves up at this time by saying the iodine will go on easily after we get the molecule to which the iodine is attached; but that is not true. The iodine will not go on easily, and I believe that this fact is very significant in some of the disturbances in thyroid diseases. We have had trouble putting the iodine on, and I think the thyroid gland has trouble in putting iodine on. If the three atoms of iodine are not attached to the molecule, the molecule cannot function normally, and a pathologic condition will result.

I will point out some of the details in the work. Starting with the thyroid gland

itself, we boiled them in nickel kettles with sodium hydroxide. It was a very disagreeable operation; at least, the clinical department thought so, because this was done downstairs below the section on nervous diseases. After boiling with alkali we added acid, which threw out a small precipitate which contained the thyroxin that had not been destroyed. The gland contains about one part of thyroxin in ten thousand parts of gland. It is a very extravagant and expensive method to isolate it, and today one milligram costs thirty-five cents—three hundred dollars a gram. We hope synthetically to make it for about thirty cents a gram.

After isolating this substance, of course we were intensely interested in finding out its physiologic effec. A child ten years old with myxedema was treated with thyroxin. She grew four inches in six months, and has continued to develop. Between January 14, 1915, and January 30, 1919, she increased 81 per cent in weight and 43 per cent in height. Her improvement in mentality corresponds. When she came ten years ago she brought her doll with her. She was about the mental age of four, but she has since gone to school, and she is now nearly twenty-one years old.

After establishing the physiologic importance of thyroxin, we were most interested in the chemical reason for its activity. What was there in that molecule that had such a tremendous effect? There are only fifteen milligrams; that is, about one quarter of a grain, in the entire human adult, and yet that amount means the difference between a high grade myxedema and a normal human being. After establishing its formula, we sought the clue to its activity, but as long as we worked with thyroxin we did not uncover the secret. I doubt that we ever would have found how thyroxin works if we had used only thyroxin.

The chief reason for the attempt to synthesize thyroxin was to explain how it functions. We started in to make it synthetically, feeling sure that before we finished we should know all there was to be known about the molecule, because when it is divided up and then put together in different sections the effect of each section is easily seen. I am not going to bore you with how we went about synthesizing it, except to point out that the compound which we started out to make was to have a formula which we ourselves had already determined—that is to say, the compass for laying our course was made by ourselves, and if that compass eventually proved to be wrong, then practically all of our work would have been of no avail. For this reason we tested the intermediate products as they were secured, in order to determine which ones would possess physiologic activity. We found that all the compounds could be divided into three groups; those which produced immediate action; those possessing no physiologic action, and those which possessed a delayed but long-continued physiologic action. In all those possessing immediate physiologic action there was one arrangement of the molecule which was the same, and this brought out the fact these compounds can exist in two conditions, an oxidized form and a reduced form.

This brings up the question of physiologic activity of thyroxin in the animal organism. A few years ago, Wieland, of Germany, suggested that oxidation was not the building up of oxygen to a superoxidase, but that really the reverse took place, and hydrogen was first broken off. Some compounds were found which would take up hydrogen very readily. Now, we feel sure tonight that thyroxin is one of that group of substances. They are called hydrogen acceptors. They increase the rate of oxidation in the animal organism, acting as catalysers between the compounds to be burned and oxygen, and carrying hydrogen as an intermediate step. If the action of thyroxin lies in the taking up and breaking off of two atoms of hydrogen from the molecule, one might ask what good is the iodine? It is only recently that we have come to appreciate what the iodine in thyroxin does. The iodine is two-thirds of the weight of the molecule, and it dominates the activity of the entire molecule. It does not break off, but stays attached to three atoms of carbon. This is what we picture as the steps which occur when thyroxin functions. As it is put in the body, the pyrrol ring immediately opens. That we can prove in a test tube. Before it can act, the ring must close, and that is brought about by the physical state of the cell in which it functions. It does not close until forces act and close it, and those forces are acting through the nitrogen in the molecule. I cannot go into the details of that, but we know that it closes, and we know it must close before it can act. When it is closed,

the molecule can be oxidized and reduced, which produces a physiologic response in that particular cell. I shall not go any further into that theory, but we have a series of compounds which will prove or disprove that theory, and we have done enough to feel sure that those steps represent the way thyroxin really functions.

If we can feel sure that the function of the thyroid is to make hydrogen burn more easily, we have assigned a definite chemical action to one of the ductless glands, and it is the first gland which has been assigned a definite chemical action. Now, if we can say that the thyroid has to do with the burning of hydrogen, then with the same terminology, what does adrenalin do, what has the pituitary to do, and what has insulin to do? When these last questions are finally answered we will have a rational explanation of the function and mode of operation of the ductless glands, based on the series of chemical reactions initiated by their presence in the animal organism.

Doctor Rowntree.—As internists, our laboratories are our workshops. In them we attempt to determine the nature, and, by quantitative measurements, the degree of deviation from normal which occurs in disease. This leads us into many fields, such as physiology, chemistry, physics, and bacteriology, and clinical and scientific work become so intermingled that it is almost impossible to draw a line between them.

Dr. Sanford's talk has been extremely interesting to me. He has discussed the more important routine laboratory examinations and has given you his impressions based on wide experience. For many years, Dr. Sanford has had charge of all the routine laboratory procedures for a very large number of clinicians. I find his conclusions of great assistance in evaluating the various routine methods employed in medicine. Each of the tests discussed, and now considered routine, represents some individual investigation, and in reality, in considering routine tests, he discussed the successful researches of the last decade or two.

One great function of this institution is the training of men. The origin and development of the Mayo Foundation is interesting. Here we have two great practical surgeons, who with a number of associates, have developed a great institution which serves not only this part of the country, but the country as a whole. As they have grown older, they have experienced what so many have experienced in the past, the feeling that financial returns alone are not satisfying or wholly gratifying, and in recognition of the need for further progress in medicine, have established the Mayo Foundation for the promotion of medical education and research. As a result, we have here at the present time 175 graduate students, young men who have just finished their internship, and are seeking further training in medicine. These men are supported by the Mayo Foundation, which is generously endowed. This indicates how the minds of the practical surgeons may run to the support of medical education and research, and of scientific progress in medicine. The Foundation is a part of the University of Minnesota, and serves, as its name indicates, two great functions: medical education and medical research.

In the development of the medical side of this work I have been tremendously interested. It offers an exceptional opportunity. We have approximately fifty Fellows in medicine and the medical specialties. The training of these men is one of the primary functions of the institution. How are we going about their training? The first requisite, in most instances, is to make of these men good clinicians. If a physician is to be a good clinician, he must first become interested in sick people and their care. He usually obtains a good start along these lines during his internship, but still needs much additional training. As a rule, the first thing is to place him in the Clinic for clinical training on a modified apprentice system. On the floor of the Clinic, he obtains this in a way that is difficult to surpass. He may also see other phases of clinical work in some of the hospital services. As a rule, after a year or so of training, he comes to one of the hospital services, or he may select laboratory service. In certain hospital services the morning is devoted to the clinical side of the work, and a part or all of the afternoon to laboratory research. Broadly, we attempt to train the graduate student clinically, and at the same time to interest him in medical research in the Clinic, in the wards, and in the laboratories.

A graduate student, who has a particular problem in which he is interested, is always given the opportunity to work on it, provided this is feasible. Others ask for problems,

or may be assigned them by members of the permanent staff. Concerning the capacity of these men to handle research problems we have learned much. Generally speaking, for the first three months, the simpler the problem, and the more concrete, the better.

Certain considerations are fundamental to the development of clinical investigation. First, it is essential to have a problem that is definite; second, methods must be utilized that are capable of solving it; such methods may not exist, and so it may be necessary to devise one; third, adequate records must be kept; fourth, the results must be interpreted and correlated with existing knowledge. These seem very simple things. However, the majority of men starting investigation fail to grasp some or all of these simple principles.

Many men come to us with the idea of picking up crumbs from this or that service. They want to be spoon-fed, or to flit from flower to flower. As a rule, they accomplish little. In research, men of this sort are usually interested only in obtaining material for a thesis, so that they may have an advanced degree. Other men come with definite, well-thought-out problems, which they wish to solve. They wish to be clinicians and investigators. For instance, we have at present, a man who wishes to spend some years of his life working out questions relating to body temperature. Obviously he has an infinite number of baffling problems facing him in the field. But since he has ideas, he will have the opportunity to work on some of them at least.

We are eager to see our men get results, but research is a good deal like farming. If a man is to produce in medicine, he must sow and cultivate before he can harvest. In other respects, it is like banking. A man may invest his time foolishly in investigation and waste it, or invest it wisely and enjoy real dividends.

Of course, the attitude that demands practical results is not the best. In addition to the great funds back of the Foundation, we have abundant clinical material which is unsurpassed anywhere, and a large group of clinicians interested in the care of the sick of today, as well as the welfare of the sick of the future. Research should not be confined to problems of the next generation, but should endeavor to solve the underlying problems of the present. The latter loom large in the minds of most of us here. How can we solve practical problems that we meet from day to day? The material for clinical investigation is here already, and it is our function to undertake problems as they are presented. Last month a leading clinician from the East stated that in his opinion one of the strongest features of our position in Rochester was the tremendous inspiration and stimulation which we derive daily from our clinical contacts. If we have any of the spirit of investigation, it is impossible to spend the morning seeing cases of unusual interest without being inspired to work out something which will throw light on the unknown, particularly with so large a group of young men who are keen to take up problems, the details of which we may not have time to undertake.

A word or two about some of the investigations in progress at present may not be amiss. In the field of diabetes and carbohydrate metabolism, Dr. Wilder and his associates have been carrying on extremely interesting investigations, which he will probably discuss with you this morning.

In the cardiorenal vascular field, Dr. Keith has been investigating the subject of dehydration, and finds that by employing sugar solution in a little less concentrated form than that employed by Woodyatt, it is possible to dehydrate the blood to the extent of 20, or even 40 per cent, without inducing any great increase in temperature. Dr. Keith, and Dr. Wagener of the ophthalmologic department, have, during the last year, observed from twenty to twenty-five cases of malignant hypertension of a type which is not commonly recognized. The patients had good renal function, by all tests employed, but marked hypertension and definite eye ground changes which are more or less pathognomonic in appearance in some instances. It is important to recognize this group, for many have died within the year, and all from vascular accidents.

Dr. Keith and Dr. Barrier are working on the treatment of edema with calcium. Ammonium chloride is also being used in the same connection. Miss Whelan, Dr. Barrier and Dr. Keith have administered various salts intravenously, and have studied the effects of various inorganic salts on the level of the anions and cations of the blood and urine.

Large doses of sodium rob the body of potassium, and large doses of calcium rob it of sodium.

Dr. Brown and his group have been studying capillaries of nail folds in health and disease. Several hundred cases have been observed, and three or four pathognomonic types of capillaries recognized. Calorimetric studies, by means of a special calorimeter devised by the Department of Physics, are also in progress in relation to the blood flow in the extremities in various vascular diseases.

Dr. Hench has developed the salivary urea index of renal function, which is probably familiar to most of you. He is also making a study of the excretion of mercurochrome, and finds that it is concentrated in the gall bladder.

Dr. Bryan is making a study of hippuric acid synthesis in kidney diseases. His findings confirm those of Kingsbury, and he has also shown that in this synthesis, the liver does not have any direct part in the mobilization of glycocol, although it may have an indirect effect.

In Rochester, we are attempting to develop group investigation analogous to our group practice. In this connection we are carrying on at the present time studies of liver function, investigations in which Doctors Greene, Walters, Snell, Counseller, McVicar and Eusterman are collaborating. In the field of jaundice we have already obtained data of very considerable clinical importance. In the next decade we may witness developments in the field of liver function comparable to those relating to renal function during the last decade. At present investigations are concerned with the value and significance of liver functional tests, rather than with liver function *per se*, a stage which we passed through in the field of renal function studies ten or fifteen years ago.

In closing, I would again lay due emphasis on the incomparable opportunity for clinical investigation offered by this institution. This opportunity, as do all opportunities, carries with it responsibilities. Our laboratories are concerned with the welfare of the sick of today, as well as with those of the future. We must remember that the routine of tomorrow rests on the successful research of today.

Dr. Brown.—An interesting resolution relative to postmortem technic was presented to the Rochester Medical Association at Rochester, New York, by the local undertakers' Association recommending that while doing an autopsy the large vessels be ligated low enough to permit the undertaker to embalm the body satisfactorily. Acting on this recommendation all pathologists in Rochester are carefully tying off all the large vessels before severing from the aorta.

There is one point which was brought up by Doctor Robertson on the sentimental side of relatives of deceased persons that has another side as I see it. A medical student who goes through his four years in a recognized medical school is able to visualize the entire picture of a postmortem examination, and it so happens in his case that in the event of the death of one of his relatives he is able to picture to himself the complete postmortem examination in all details as the request is made for permission. If the examination is made he is really subjected to a double grief and it seems to me it is rather hard on some types of trained medical men to ask them to suffer twofold unless the case warrants it. Now, the layman does not have to go through quite the same experience; he has in his mind no picture of what is to take place and he suffers from the death of the relative alone.

A friend of mine told me of an autopsy carried out in an undertaking room several years ago that shows how a postmortem examination may react on a relative even after he has given his consent to the same. The day was warm and the door of the examining room was left open though unfortunately it was not guarded. The relative paced back and forth on the walk and finally worked around to the back of the building and casually looked in through the door and saw the operator at work on the autopsy that to him was most gruesome. The shriek that came from his lips was that of extreme agony and pain and one that none of us would care to hear. Here of course the whole plan was wrong but it brings to our attention the need of the greatest care in the conduct of our postmortem studies.

I think we have got to be careful and tactful, considering the general conditions and

obtain permission on first, the cases dying of causes unknown, second, on the doubtful or partly obscure cases and lastly on the routine cases with clean cut diagnosis of the causes of death.

It seems to me that tact is most worth while in obtaining postmortem examinations and the impression should be definitely left with the relatives of the deceased whenever it is possible to do so that a favor is being conferred upon them by making the examination and that it is not being made to satisfy the curiosity of a few physicians and students. The body in the postmortem room should be treated with the greatest consideration, first as a mark of respect to the dead who was beloved by some one, sometime, and second, that the embalmer may have a chance to restore the appearance so that no observer can see the lines of incision.

The great purpose of medicine is to prevent and relieve human suffering and it seems to me that the suggested wholesale postmortem examinations would be contrary to the fulfillment of this aim and many needless routine autopsies would be made under this policy that would on completion have added not one item of interest, knowledge or satisfaction to anyone except perhaps the statistician.

The Journal of Laboratory and Clinical Medicine

VOL. X ST. LOUIS, MO., OCTOBER, 1924 No. 1

Editor-in-Chief: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - CINCINNATI
PAUL G. WOOLLEY, M.D. - - DETROIT
J. J. R. MACLEOD, M.B. - - - TORONTO
ROY G. PEARCE, M.D. - - AKRON, OHIO
W. C. MACCARTY, M.D. - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - IOWA CITY
MARSH PITZMAN, M.D. - - - ST. LOUIS
RUSSELL L. HADEN - - ROSEDALE, KAS.
JOHN A. KOLMER, M.D. - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - LOS ANGELES
A. PARKER HITCHENS, M.D. - WASHINGTON

Official Organ of the American Society of Clinical Pathologists

Contents of this Journal Copyright, 1924, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

New Light on Essential Hypertension

NUMEROUS theories have been advanced to explain arterial hypertension. It is well recognized that changes in one or more of the several fundamental factors upon which normal arterial tension depends may give rise to an increase in blood pressure. It was first thought that high blood pressure is always secondary to kidney injury and probably an adjustment to compensate for the increased force necessary to drive the blood through the diseased kidney.

The kidney factor has been perhaps overemphasized. The trend of opinion is towards a chemical explanation for the rise in pressure rather than structural kidney changes. This view is due largely to the separation, as a clinical entity, of a condition in which the arterial hypertension is the primary factor. Janeway gave to the syndrome the name, "chronic hypertensive vascular disease," while Sir Clifford Albutt designates it, "hyper-

piesia." "Essential hypertension" is the term most commonly employed. It is agreed that the fundamental change is an abnormal contraction of the arterioles.

The greatest interest in the disease after recognizing the essential anatomic basis has been the search for the pressor substance or substances responsible for the arteriolar spasm. The facts known at present concerning pressor bodies are well summarized by Shaw[1] in his excellent monograph, *Hyperpiesia and Hyperpiesis.* Soluble pressor substances are remarkably few in number. Those occurring in the body are protein in nature and may be of metabolic, bacterial, cellular, or intestinal origin.

Dale and Dixon have described in detail the pressor action of amines such as isoamylamine, p-hydroxyphenylethylamine, and phenylamine which are formed in the course of the putrefaction of protein. These, it is supposed, may be formed in the intestinal tract of man, or possibly by prolonged peptic digestion. Tigerstedt, Bergman and Shaw, have also shown that an extract of the normal kidney cortex, known as renin, will produce a temporary rise in arterial pressure on intravenous injection.

It has not seemed possible to critical observers, however, that any of the pressor substances heretofore described can be responsible for essential hypertension. The pressor action has usually been of a minor degree and evanescent. In most instances the pressor substances have been abnormal products of metabolism. There has been no proof of the presence in the body of the substances which have been demonstrated experimentally to have a pressor action.

Major and Stephenson,[2] of the University of Kansas Medical School, have recently opened up a new avenue of investigation by demonstrating that at least one normal metabolite is a pressor substance to a marked degree. Beginning with the assumption that the pressor substance must be of protein origin, these workers have investigated the properties of some of the better known metabolites. They found that urea, uric acid, creatin, and creatinine are without effect on blood pressure, but that the guanidin bases have remarkable pressor effects. A dose of methyl guanidin varying from 0.1 to 0.2 gm. per kilogram of body weight will double or even triple the blood pressure of a normal dog and maintain this level often for four or five hours. A number of guanidin bases have been studied and all apparently have this pressor effect. Methylguanidin and dimethyl guanidin are normal end products of protein metabolism. Creatinine is a closely related body being methylguanidin acetic acid. Normal individuals excrete about 100 mg. of guanidin bases per day. In experimental uranium nephritis, chronic nephritis with hypertension, and in essential hypertension there is a decrease in excretion as compared with normal individuals.

The guanidin bases have been the subject of numerous investigations especially as a possible cause of tetany. In large doses they produce irregular heart action, a slowing of the pulse, and convulsions. It has been repeatedly emphasized that essential hypertension, uremia and eclampsia have much in common. The fact that the guanidin bases produce the symptoms

characteristic of these allied conditions suggests that it may be the toxic agent common to the three.

This work of Major and Stephenson constitutes probably the most important contribution made to essential hypertension in the past decade. It opens up a new field of study and offers many potential possibilities.

REFERENCES

[1]Shaw, H. Batty: Hyperpiesia and Hyperpiesis, Henry Frowde, London, 1922.
[2]Major, R. H., and Stephenson, W.: Bull. Johns Hopkins Hosp., 1924, xxxiv, 140; 1924, xxxv, 186.

—*R. L. H.*

The Scope and Function of the Laboratory Report

BROADLY speaking, there are only two methods of practicing medicine: A hasty survey, coupled with a tacit acceptance of the patient's diagnosis of "indigestion" or what not and followed by a more or less effective symptomatic prescription—a method which has been succinctly termed "peddling pills"; or an honest, scientific attempt to diagnose the underlying cause of the symptomatology and to formulate a plan of treatment in accordance with its mechanism.

There is no satisfactory or successful middle course or compromise, even though the latter method may lead through tortuous and devious by-paths before the etiology and mechanism of the associated phenomena comprising the symptoms are laid bare.

The education of the public proceeds slowly—but it proceeds and the proportion of individuals increases who realize the necessity for thorough, careful and systematic procedures directed toward the accumulation of data, the thoughtful consideration of which may lead to the formation of a diagnosis as an essential prerequisite for intelligent treatment and who, therefore, are cognizant of the existence and expect the use of certain methods of greater or less precision in the study of their condition.

Manifestations of disease, in brief, are manifestations of disturbance or loss of function and not infrequently the particular functional imperfection which, because of its interference with the comfort or habits of the patient, has led him to consult the physician is only the end of a devious path leading back to a primary cause only indirectly connected with the presenting complaint and which must be uncovered by, at times, sustained endeavor and laborious search.

Each case, therefore, necessitates a careful survey and no satisfactory or complete survey is possible in these modern days without the introduction and utilization of varied methods of laboratory examination and study, the value of which is directly proportionate to the care and appropriateness with which they are chosen and the skill and keenness with which the results are interpreted.

Unfortunately, however, in many instances the importance and significance assigned to the results of laboratory examinations is predominant and unwarranted and they are too often looked upon as something separate and

apart from all other methods of study and at times even made to replace, supplant, or render apparently unnecessary other and detailed methods of examination.

That this is true cannot be controverted when one considers the number of cases in which the diagnosis of syphilis, tuberculosis, or goiter, for example, rests almost entirely upon the results of complement-fixation tests, sputum examinations, or determinations of the basal metabolic rate; or, what is still more to be decried, such diagnostic possibilities are cast aside because of "negative" laboratory reports.

Because the refinements and elaborations of laboratory technic have, under certain circumstances and with well-defined but not always equally well-appreciated limitations, conferred a definite significance upon positive results, a more or less equal significance has been attributed to negative results without, however, comparable premises to warrant such deductions.

Because laboratory findings of various kinds are a relatively constant feature of certain pathologic entities there has been a tendency to think of such reactions as definitive of the disease in question; to assume that, if their occurrence at times constitutes weighty evidence in favor of the disease, their absence likewise is equally weighty evidence against such a possibility—forgetting that—*always* the occurrence of positive reactions depends, not only on the presence of special stimuli setting in motion the mechanism responsible, but also that the occurrence of the reaction, the detection of "positive" laboratory findings is directly dependent upon and regulated by two important factors: the varying ability of different individuals to respond to similar stimuli, and the perfection, delicacy, and reliability of the methods used to detect and measure such response.

The laboratory report and methods of laboratory examination should not, therefore, be exalted to the neglect and minimizing of other methods of examination and study, which prior to the elaboration of laboratory methods, furnished the foundation upon which the diagnostic superstructure might be erected.

It is not the function of the laboratory *ipso facto* to make the diagnosis or of the laboratory report to furnish the sole or predominant basis for diagnostic assertions. Just as the fluoroscope and the films of the intestinal tract furnish the clinician with a graphic picture of the workings of the tract under examination, so it should be the function of the laboratory report to furnish him with a graphic record from a consideration and interpretation of which he may construct a mental image of the mechanism and functional efficiency of the structures under consideration.

It is not the report, therefore, but its interpretation which is of significance and the value of the laboratory report is hence in direct proportion and indissolubly related to the care and acumen with which the method of laboratory study is planned, and the skill and keenness with which its results are weighed and evaluated *in conjunction with all the information pertaining to the case* elicited by all the methods available and of which the clinician is cognizant.

There are, at present, so many divisions and subdivisions of the laboratory, so many specialized methods of laboratory study, that it will rarely be feasible—especially in private practice—nor is it always necessary or desirable because of the financial outlay demanded of the patient, to subject every case without exception to complete laboratory studies. Procedures of great value and significance in one condition may have relatively little value in another. He who most wisely assorts his laboratory requisitions in accordance with the possibilities to be considered; who chooses them with a definite understanding of the value and significance of the results as applied to the particular case, will utilize the resources of the laboratory most wisely and efficiently and will derive far more of value from its reports than he who makes requisitions in haphazard fashion with the subconscious or tacit hope that, in some mysterious way, the results will whisper the diagnosis and obviate the necessity for other and, perhaps, tedious and time-consuming clinical studies.

In the clinical evaluation of laboratory findings it must be remembered that it is not the isolated result, the data of single examinations, but the constancy with which abnormal findings occur which, in the long run, confers the greatest significance.

Single urinalyses, agglutination tests, complement-fixation tests, or other determinations are of little value unless interpreted in conjunction with all the other circumstances of the case which may, perhaps, very markedly modify the conclusions to be drawn from them, and unless it is realized that they apply only to the single specimen examined under these circumstances and that they are of far less value and significance than the correlated fact that the apparent abnormalities so detected are constantly found upon subsequent examinations.

The isolated determination of a decreased acidity in a single examination of gastric contents suggests only the *possibility* of hyperchlorhydria, for example, but the repeated finding renders the possibility a probability to be correlated with, and perhaps corroborated by, the other available data.

The diagnosis of syphilis may, at times, be practically made by a properly performed, checked, and controlled complement-fixation reaction, but it can never be negatived with an equal degree of assurance by a single negative reaction.

The tendency to associate certain laboratory examinations with certain diseases as predominant, invariable, and pathognomonic evidence of such disease and their absence as equally good evidence of the absence of such a condition, is to be strongly decried as unwarranted by the evidence and tending toward a neglect of clinical examination and study.

The laboratory report should be merely a source of information; a means of supplying the clinician with data not otherwise available, and the data should be supplied and sought for with the distinct understanding that it is to be weighed, evaluated, and interpreted; that it is not, in itself, a complete study or examination, but merely a *phase* of such a study with which all the other information must be correlated.

Its true value, therefore, lies in its interpretation—and the correctness and significance of the interpretation is determined solely by the skill, experience, and acumen of the interpreter.

Upon whose shoulders this responsibility shall rest; whether upon the clinician to whom the report is made, or the clinical pathologist by whom it is constructed, is a matter of debate with much to be said in favor of the utilization of the attainments, experience, and skill of both upon the neutral ground of consultation.

—*R. A. K.*

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

---

*The National Health Series**

IN producing this series the publishers have made a distinct contribution toward the popularization of preventive medicine, paralleling in booklet form the valuable work of "Hygeia" which appears as a periodical. The series of twenty volumes is edited by the National Health Council and each booklet is written by a recognized authority.

"Community Health" is covered by Dr. Armstrong, Executive Officer of the National Health Council, who is well fitted to write upon this subject by virtue of his experience in directing the Framingham Experiment, In "Personal Hygiene" Dr. McLaughlin of the U. S. Public Health Service gives in a direct manner the rules for right living. He describes as clearly as one may of what true health consists. He discusses very simply the application of personal hygiene and emphasizes periodic health examinations. T. Stuart Hart discusses disease of the heart and circulatory apparatus. Lucy H. Gillette in another volume writes upon practical dietetics. In a volume entitled "The Human Machine" Dr. William H. Howell contributes a section on practical physiology.

Jas. A. Tobby, Administrative Secretary of the National Health Council has written a story of "The Quest for Health." Following the high road, he points out the misleading byways, calls attention to the helpful guide posts placed along, and lead us to the end of a pleasant journey. Richard A. Bolt, of the American Child Health Association, writes on babies and Henry L. K. Shaw of Albany Medical College on "The Young Child's Health, his Normal Growth and development, and Problems of Hygiene and Training."

"Cancer, Its Nature, Diagnosis and Cure," is covered by Francis C. Wood, Director of Cancer Research of Columbia University. He follows in general the lines that are emphasized by the American Society for the Con-

---

*National Health Series, Edited by the National Health Council. Funk & Wagnalls Company, New York and London, 1924. Twenty Vols. Flexible fabrikoid. Price per set $6.00, per volume 30 cents net.

trol of Cancer and incorporates a chapter upon cancer quacks. Dr. C. E. A. Winslow is eminently qualified to discuss communicable diseases.

Each volume is a vest pocket edition with flexible leather covering and may be read in spare moments on the street car, in the train, or while awaiting an appointment. The price is within the reach of all, and the binding is so attractive that the books may well be displayed as a decoration for the library table. All volumes are of approximately equal length. We regret that some are not more detailed, for justice cannot be done to certain of the subjects in the small space allotted. This applies particularly to the contributions on dietetics and on child health.

The medical profession as a whole should become thoroughly acquainted with these volumes and with similar contributions, such as those found in "Hygeia," lest they soon find the wide-awake layman better acquainted with recent developments in the fields of life extension and public and individual health than he is himself.

---

*Protoplasmic Action and Nervous Action**

A TREATISE on the physico-chemical basis of the more general or fundamental properties of living matter. The author approaches the problem from a physico-chemical basis. He feels that the regularity of the living organism presupposes regularity of its component physico-chemical processes. In fact, one of the most striking features of organic processes is their exactitude, which is frequently safeguarded by regulatory devices of the utmost delicacy. The volume contributes a distinct addition to our knowledge of biologic processes, particularly those of irritability and the transmission of stimuli through living matter.

Epitome on Blood Pressure†

A COLLECTION of disjointed statements with reference to blood pressure, most of which are based on existing knowledge. The pages devoted to treatment discuss the value of autocondensation. In fact this appears to be a brochure on autocondensation in the treatment of hypertension. The cost of the book is surprisingly high.

---

*Protoplasmic Action and Nervous Action. By Ralph S. Lillie, Biologist, Nela Research Laboratories, Cleveland; formerly Professor of Biology, Clark University. Cloth. Price $3.00. Pp. 417. University of Chicago Press, Chicago, 1923.

†Epitome on Blood Pressure. By Burton Baker Grover, M.D. Author of "Handbook of Electrotherapy" and "High Frequency Practice"; President of the Western Electrotherapeutic Association, 1919-1921. Pres. of the Western School of Physiotherapy. Paper. Price $3.00. The Electron Press, Kansas City, Mo.

The American Society of Clinical Pathologists

Dr. John A. Kolmer, Philadelphia, Pa.
President

Dr. Frederick E. Sondern, New York
1st Vice President

Dr. W. F. Thomson, Beaumont, Texas
2nd Vice President

Dr. Ward Burdick, Denver, Colorado
Sec'y-Treas.

Executive Committee:
Dr. Murray G. Stone, Springfield, Mo.
Dr. Herman Spitz, Nashville, Tenn.
Dr. A. H. Sanford, Rochester, Minn.

Board of Censors:
Dr. Willis P. Butler, Shreveport, La.
Dr. A. H. Schade, Toledo, Ohio
Dr. Ernst A. Victors, San Francisco, Cal.

The A. S. C. P.

The A. S. C. P. in its brief period of existence has already rendered great service to the cause of scientific medicine by helping to elevate the status of the clinical pathologist in his relation to the clinician and to stimulate the use of laboratory aids in diagnosis.

Older and long established societies may be quiescent during a whole year and only show signs of activity at the annual meeting. The American Society of Clinical Pathologists, however, is a young, vigorous and actively growing organization with strong proliferative tendencies aiming to keep the members constantly in touch with one another and spurring them on to better scientific and technical perfection of their specialty.

Membership in the A. S. C. P.

The secretary of the A. S. C. P. reports a flood of inquiries regarding membership in this national body. The clinical pathologists throughout the country are recognizing the benefit of organization and appreciate the excellent results achieved by the A. S. C. P.

Application blanks and information may be obtained from Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado.